PHARMACEUTICAL STUDIES OF EPIRUBICIN EMULSION

by

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ABSTRACT

Epirubicin has been used as an anticancer agent in the treatment of inoperable hepatocarcinoma in which case it is administered as an aqueous solution intravenously after reconstitution of the factory-packed dry powder. Pharmacokinetic analysis of the current method of mixing the aqueous solution directly with Lipiodol, an oily contrast medium, and injecting the mixture intra-arterially does not reveal any significant clinical superiority over the aqueous solution, although some degree of targeting effect was suggested. This could probably be due to a lack of pharmaceutical consideration and technology in the formulation of the preparation.

An oil/water emulsion containing epirubicin for parenteral administration has been developed with due consideration given to basic principles of pharmaceutics and formulation. The stability of the emulsion, sterility, rate of drug release, toxicity, and pharmacokinetics after administration into rabbits were studied and reported.

Extraction of epirubicin from various media was performed by a modified assay based on earlier reports. Determination of the drug and its metabolite in plasma samples was carried out by high performance liquid chromatography. Different pharmacokinetic parameters, obtained from plasma

concentrations using computer programmes, were used to compare the disposition of epirubicin after intravenous and intrahepatic injections of the emulsion and solution of the drug.

Findings indiate that since a lower area under the curve (AUC) was obtained after intrahepatic injection of the formulated emulsion, a targeting effect is possible. It may therefore enable intrahepatic administration of larger doses of epirubicin with perhaps less systemic side effects.

CHAPTER I: INTRODUCTION AND SOME DISPOSITION PRINCIPLES

Since the early 1980s, many research projects have been devoted to the special formulation of antineoplastic agents for the treatment of hepatocarcinoma (1 - 9). One area of interest is the use of special formulations together with a selected route of administration in order to obtain a targeting effect on the tumour. However, results have not been too encouraging in terms of clinical improvement and reduction in side effects.

A review of the previous reports shows a possible reason for unsatisfactory results : a lack of consideration of pharmaceutical formulation in the development of the solutions. Many factors should be considered when an oil is added to an aqueous solution of anticancer agents in the design of an emulsion.

In the present study, we are interested in developing an emulsion for intra-arterial injection in order to obtain a targeting effect on liver tumour cells. In view of past experience, most of this thesis is devoted to the formulation of an emulsion based on pharmaceutical principles.

In this study, a lipid contrast medium for lymphograph, Lipiodol, was chosen to be the oil phase of the emulsion due to its unique property of preferential uptake by hepatoma cells and retention for a prolonged period of time after intrahepatic injection (10, 11). It was mixed with an aqueous solution of epirubicin, an anticancer agent, and formulated into an emulsion with the use of emulsifying agents.

EPIRUBICIN

Epirubicin is a potent antineoplastic agent of the anthracycline group. Pharmacologically it is classified as an antibiotic antineoplastic agent. Its structural analog, doxorubicin (Adriamycin), has already been shown to be effective in treating hepatoma at the cost of a considerable degree of cardiotoxicity.

Anticancer activity of epirubicin is due mainly to its affinity for DNA, with intercalation into the DNA double helix structure (12, 13). This complexing

with double-stranded DNA results in disruption of nucleic acid synthesis and function, due to inhibition of the biochemical processing of template DNA by DNA polymerase (14).

Currently, epirubicin is indicated in the following neoplastic conditions (15):

1. mammary carcinoma
2. malignant lymphomas
3. soft tissue sarcomas
4. gastric cancer

Preliminary evidence of efficacy has been found in malignant melanoma and advanced colorectal carcinoma. When given in combination with other cytotoxic agents, it is also found to be able to induce therapeutic response in lung and ovarian cancers (15).

Although hepatocellular carcinoma is not yet a proven indication for epirubicin therapy, preliminary evidence has shown some degree of therapeutic response (9, 16, 17).

Pharmacokinetically, epirubicin has uptake and distribution comparable with doxorubicin, yet with a lower tissue concentration, suggesting an increased hepatic metabolism and/or a higher renal clearance of epirubicin (18). Metabolism of epirubicin gives several metabolites which can be detected in plasma and urine after administration , the major one being 13 epidoxorubicin (13 - OH). Due to low water solubility of the free base, epirubicin is currently supplied in the form of hydrochloride salt for intravenous administration.

A number of techniques for the detection of epirubicin and its metabolites in plasma have been reported in the literature (19, 20), but high-performance liquid chromatography (HPLC) with fluorescence detection appears to be the best method as it is reproducible and can also differentiate between epirubicin and its 13 - OH metabolite in plasma (21, 22).

EMULSION THEORY

Emulsions are defined as heterogeneous systems of one liquid dispersed in another in the form of droplets. The two liquids are immiscible, chemically unreactive and form systems characterized by little to no thermodynamic stability.

When two immiscible liquids are mechanically agitated, both phases tend to form droplets which greatly increase the free surface energy of the system, and at such a high energy level the system becomes unstable. Through the process of coalescence this free surface energy again attains its minimum value. If a stable emulsion is to be formed, an emulsifying agent (emulgent/emulsifier) must be added, which by its presence at the interface prevents coalescence of the globules. It acts by reducing the interfacial tension and hence decreasing the interfacial free energy produced on dispersion. Another more important role to act as an interfacial barrier which, by being present in sufficiently high concentration, will

produce a rigid film between the two immiscible phases. The film may thus act as a mechanical barrier both to flocculation and to coalescence of the emulsion droplets.

The material that is dispersed into small particles or exists as globules is termed the internal, disperse or discontinuous phase. The other material is known as the external phase, continuous phase, or dispersion medium.

The most common types of pharmaceutical emulsion include water as one of its phases and an oil or other liquid as the other. If the oil droplets are dispersed in a continuous aqueous phase, the emulsion is termed oil-in-water type (o/w), if the oil is the continuous phase, the emulsion is of the water - in - oil type (w/o). The oil and water phases need not be single components, each phase may contain a multitude of ingredients. Most pharmaceutical emulsions designed for oral administration are of the o/w type, while externally used emulsions such as creams and lotions are either o/w or w/o depending on their use. For the purpose of the present study, we shall concentrate on emulsions for parenteral use.

Emulsions for parenteral use are usually o/w type, especially when they are for intravenous or intra-arterial use, to avoid the risk of thromboembolism. An oil soluble medicament is dissolved in a suitable carrier and the mixture is emulsified to form the final preparation. However it is essential, but relatively difficult, to achieve stable droplets of less than 4 um in order to prevent emboli in the blood vessels (23). Another limitation on injectable emulsions is the requirement for sterility, as conventional bactericides are contraindicated in large volume parenteral injections, and autoclaving has been the traditional method of sterilization for parenteral emulsions. Regardless of the limitations, a number of such emulsions have been designed and used clinically. Intravenous fat emulsions using lecithins and non-ionic surfactants as emulsifying agents are the most commonly used examples.

Although oil soluble drugs are usually incorporated in the oil phase of an oil-in-water emulsion for parenteral use, there have been studies showing that water - soluble drugs can also be administered in emulsion form, resulting in enhanced absorption (24, 25). This is probably because a portion of the drug is bound to the oil droplets.

Stability has always been the major concern in the formulation of an emulsion. Instability may appear as one or more of the following situations:

a. Creaming

This is the separation of the emulsion into two layers, one layer containing more of the disperse phase. The layer can be redispered by shaking. It can be avoided by reducing the size of globules and increasing the viscosity of the continuous phase.

b. Cracking

This involves the irreversible coalescence of the dispersed globules and separation of the disperse phase as a separate layer. Redispersion cannot be achieved by shaking and the preparation can no longer be used. It may result from using the incorrect combination or proportion of emulsifying agents, chemical or microbial degradation, addition of a common solvent, incorrect method of addition of electrolytes, improper storage temperature, and significant changes in pH.

c. Phase inversion

Phase inversion occurs as the amount of disperse phase is increased until it approaches or exceeds the theoretical maximum of 74% (37) of the total volume, when the emulsion changes from o/w to w/o or from w/o to o/w type.

SOME PRINCIPLES OF DRUG DISPOSITION

The analysis on the disposition of a drug is mainly based on the pharmacokinetic parameters obtained from analyses. Unlike pharmacology, the subject of pharmacokinetics describes, expresses and predicts the absorption, distribution, metabolism and elimination of a drug using symbols and mathematical equations. It is therefore a science of the relationships between the movement of a drug through the body and the processes affecting it.

The body can be viewed to be composed of a very large number of compartments. A compartment, in pharmacokinetic terms, is those organs and tissues for which the rates of uptake and subsequent clearance of a drug are similar.

For the purposes of this study, only expressions and relations describing non-compartment models are discussed.

1. Bolus intravenous injection

When a rapid single intravenous injection (a bolus) is given to a patient, if we plot plasma concentration against time, we will find the concentration declines rapidly at first and then more and more slowly, and eventually comes very close to zero. (Fig (1) - 1)

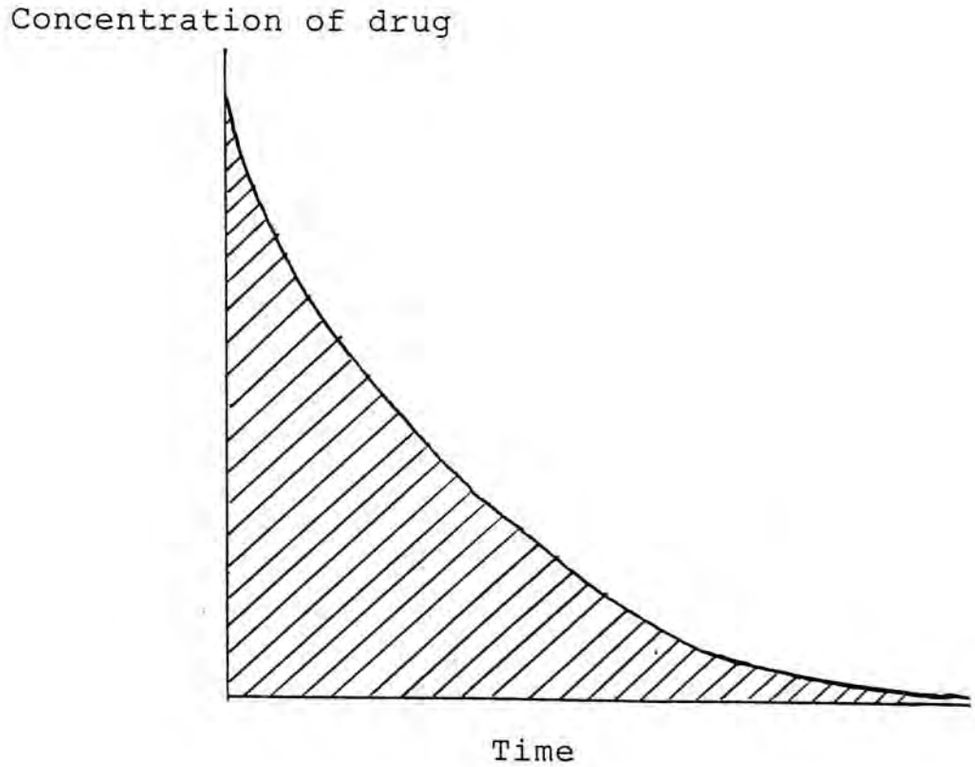


Fig (1) - 1

The phenomenon can be described by the following equation:

$$D = D_o\, e^{-kt} \qquad \text{Eq. (1)}$$

where D = amount of drug in the body at time t

D_o = initial amount of drug (the initial dose)

K = elimination rate constant

Thus, the rate of elimination of the drug varies continuously depending upon its concentration at any given time (31).

2. Volume of Distribution/Apparent Volume of Distribution

The volume of distribution (Vd) of a drug does not refer to any real volume (31). It is simply the size of a compartment necessary to account for the total amount of drug in the body if it were present throughout the body in the same concentration found in the plasma. Mathematically, it can be expressed as

$$Vd = \frac{D}{Cp} \qquad \text{Eq. (2)}$$

where D = total amount of drug in the body at time t

Cp = plasma concentration at time t

Apparent volumes of distribution which are larger than the actual plasma compartment (i.e. greater than 3 litres) only indicate that the drug is also present in tissues or fluids outside that compartment (31). The actual sites of distribution cannot be determined from this value.

The apparent volume of distribution is a function of the lipid versus water solubilities and of the plasma and tissue protein binding properties of the drug. Factors which tend to keep the drug in the plasma or increase Cp, such as low lipid solubility, increased plasma protein binding, or decreased tissue binding, reduce the apparent volume of distribution. It follows then that factors which decrease Cp, such as decreased plasma protein binding, increased tissue binding, and increased lipid solubility increase the apparent volume of distribution.

3. Bioavailability and Area Under the Curve (AUC)

Bioavailability is the degree to which a drug is made available to a specific organ or tissue different from the site of absorption for a pharmacological response to occur. It is therefore apparent that the intensity and duration of the tissue response is probably in most cases a function of the concentration and persistance of the drug in the blood or plasma.

Figure (1) - 1 represents a typical plasma concentration versus time plot for a drug administered intravenously. The area under the curve (AUC) i.e. the hatched area in Fig (1) - 1, is a useful measure of the amount of drug absorbed into the systemic circulation (see Page 10).

We would therefore expect the size and duration of the biological response to be related in some way to the size of this area. In other words, a measure of this area can be a useful index of the bioavailability of the drug. The AUC_0^{∞} from time zero to infinity can be obtained from Equation (3)

$$AUC_o^{\infty} = AUC_o^{t} + \frac{C_p^{t}}{K} \qquad \text{Eq. (3)}$$

where Cp^t = plasma concentration at time t.

K = elimination rate constant

4. Clearance

Clearance is the volume of blood or plasma cleared of drug in a unit time. It is obvious that clearance must be related in some way to the volume in which the drug is dissolved (volume of distribution Vd) and the rate at which it leaves the body (i.e related to the elimination constant K). If a drug is cleared from the plasma by several processes then the total clearance is the sum of the individual clearances. Plasma clearance is expressed by the relationship

$$Cl = V_d K \qquad \text{Eq. (4)}$$

Clearance is the sum of individual clearance values (31)

$$Cl_P = Cl_M + Cl_R + Cl_H + \ldots \quad \text{Eq. (5)}$$

Where Cl_P = plasma clearance

Cl_M = metabolic clearance

Cl_R = renal clearance

Cl_H = hepatic clearance

Because plasma clearance is the product of elimination rate constant and volume of distribution (From Eq. (4)), we would expect it to be closely related to the AUC (31).

$$Cl = \frac{Dose}{AUC_0^{\infty}} \quad \text{Eq. (6)}$$

5. Half - life ($t_{1/2}$)

The half - life is the time taken for the concentration of drug in the blood or plasma to decline to half of its orignial value. It is

given that

$$t_{1/2} = \frac{0.693}{K} \qquad \text{Eq. (7)}$$

Apart from the above, other parameters include the mean residence time (MRT) which is calculated from the ratio of AUMC/AUC where

AUMC = area under the first moment curve (31).

Also the steady state apparent volume of distribution (Vss) can be calculated from equation (8).

$$Vss = Clp.\ MRT \qquad \text{Eq. (8)}$$

All these principles will be used in the subsequent analyses of the different studies. The pharmacokinetic parameters presented will be computed by the programme PKCALC (32). Significance of difference of kinetic parameters among the different treatment groups are evaluated using the Student's non-pair t-test, with $P < 0.05$ considered to be the limit of significance.

CAHPTER II : DETERMINATION OF EPIRUBICIN IN BIOLOGICAL FLUIDS

1. INTRODUCTION

Different methods have been developed for the assay of epirubicin in plasma (33, 34, 35). The experiments done by Moro et al in 1981, though sensitive and efficient, required the process of evaporation under reduced pressure (33), and hence were more time-consuming and expensive. Later efforts incorporated an extraction procedure, and a minute amount of desipramine was added into the back-extracting solution to avoid adsorption losses. However the retention times for epirubicin and its metabolite were relatively long requiring about 22 and 18 minutes respectively (33). Also, no internal standard was included in the assay making the results questionable (34). Likewise, the method developed by Maessen et al in 1987 had similar long retention times (about 14 minutes for all compounds) in addition to irregular recoveries ranging from 49-75% (35). The assay developed for this project was based on a modification of the above methods.

2. METHOD

A. Apparatus

The chromatography system comprises of a Waters 501 pump (Waters - Associates, Milford, Mass, U.S.A.), a 7125 injector (Rheodyne. Calif, U.S.A.) and a variable wavelength Hewlett-Packard HP1046A programmable fluorescence detecter (Hewlett-Packard, U.S.A.), and the results were recorded on a Linseis L6512 chart recorder (Linseis, Germany). Analyses were performed on a Nova-Pak reversed phase C18 column (100mm x 8mm I. D., Waters Associates, Millipore, U.S.A.) held in a Waters RCM cartridge holder (Millipore, U.S.A.) linked to a C_8 precolumn (packed with Perisorb RP-8 30-40um, E. Merck, Germany). Centrifugation was done in a Hitachi HIMAC SCR 18B centrifuge (Hitachi, Japan).

Other apparatus include 10ml capacity centrifuge test tubes with well fitting screw caps (Pyrex, England), and 15ml evaporation tubes with finely tapered bases of 100ul capacity (Sovirel, Levollois-Perret, France).

All test tubes were silanised by rinsing with a 3% solution of hexamethyldisilazane (HDMS) in distilled chloroform to minimize loss of drugs to adsorption onto the glass wall. All glass wares were cleaned by soaking overnight in a 3% solution of Extran (Merck, Darmstadt, Germany) in water, then rinsed thoroughly with methanol and hot tap water followed by distilled water.

A 25ul Hamilton microsyringe was used during the extraction procedure while a 50ul one was used for injection into HPLC.

B. Materials

The following materials were used : 3-propanol, chloroform, sodium hydrogen phosphate and ortho-phosphoric acid, all of analytic-reagent(AR) grade (Merck, Germany). Water was double distilled in a glass apparatus. Epirubicin and doxorubicin were purchased from the agent of Farmatalia Carlo-Erba in Hong Kong in vials of 10mg for parenteral use while the metabolite, 13 - epidoxorubicin (13 - OH),

was supplied by the original manufacturer. Lipiodol was also purchased from the agent of Guerbet (France) in Hong Kong. Other materials used include polysorbate 20 (Tween 20), sorbitan monolaurate (Span 20), poloxamer (Sigma, U.S.A.) and acetonitrile (AR) grade (Merck, Darmstadt, Germany).

C. Chromatographic Conditions

The mobile phase consisted of acetonitrile 35% in 0.05% phosphoric acid, with pH adjusted to 3.5. The column was washed after each assay with acetonitrile 60% in double distilled water. Every time before use, both the mobile phase and the washing solution were filtered using a 0.45um Millipore membrane (Waters Associates, Milford, Mass, U.S.A.). Filtration was assisted by a vacuum pump.

Flow rate was adjusted to 2.2 ml/min. The fluorescence detector was set to the following conditions : excitation wavelength at 470 nm, emission wavelength at 580nm, peak gain at 11 and temperature of assay is at room temperature

(about 25°C). The concentrations of epirubicin and 13-dihydroepirubicin (13 - OH) were calculated using the peak height ratios relative to the internal standard.

D. Sample Preparation

Plasma samples were obtained from the rabbit as described in Chapter IV. Sample volumes ranging from 0.6 to 1.0 ml were pipetted into 10 ml centrifuge test tubes with screw-cap. If less than 1.0 ml, the volume was made up to 1.0 ml with double distilled water in each case. 15 ul of doxorubicin 100 ug/ml was used as the internal standard. One millilitre of sodium hydrogen phosphate (1.18 G/100ml, adjusted to pH 8.4) was added and the mixture was mixed by vortex mixer. Epirubicin was extracted by adding 4 millilitres of a mixture of chloroform: 2-propanol (3:1). The whole mixture was mixed well with a rotating mixer for 30 minutes, and then centrifuged at 3000 revolutions/minute at 20°C for 15-20 minutes. Afterwards the top aqueous layer was discarded by pipette and the

bottom organic phase was transferred to a clean centrifuge tube. The drugs were back extracted by 0.15 millilitre of 0.1 M ortho-phosphoric acid with 10ug/ml of desipramine to avoid loss due to adsorption on glass wall. The mixture was mixed by a rotating mixer for 30 minutes, then centrifuged for 15 minutes. Afterwards the top aqueous layer was transferred by pipette to an evaporating tube and was again centrifuged for 15 minutes, 100 ul of the resulting solution was injected into HPLC for analysis.

All compounds were eluted within 6 minutes. The whole extraction procedure required approximately 6 hours for a batch of 26 samples.

E. Calibration and Recovery

Blank plasma samples were spiked with known amounts of both epirubicin and 13-dihydro-epirubicin to give concentration ranges of 125, 250, 500, 1000 and 2000 ng/ml. The samples were treated, extracted and analysed by HPLC as described above. Six calibration curves were

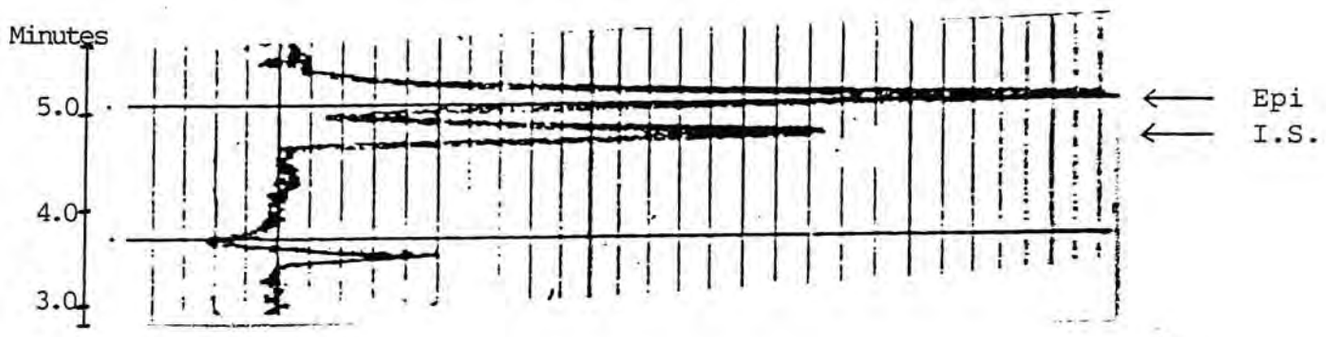
drawn by plotting mean peak height ratios of epirubicin and 13-dihydro-epirubicin to that of internal standard against the respective concentrations. Recovery was assessed by comparing peak heights in the chromatograms of extracted samples with those samples containing known concentrations of the drugs but without undergoing extraction procedure. In both cases, internal standard was only added just before injection into HPLC.

F. Results and Discussion

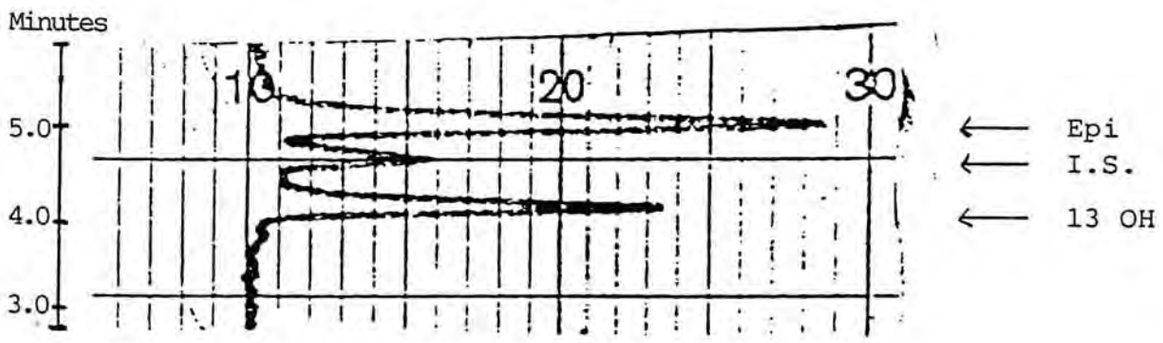
The retention times for 13 - OH, internal standard and epirubicin are 4.2, 4.8 and 5.2 minutes respectively. The representative chromatograms of the compounds in standard solution compared to those in a sample extract are shown in Fig (2)-1.

The detection limit of the assay ranged from 125 ng/ml to 2000ng/ml, which compares favourably with other assays (33-35).

The recoveries of the compounds from spiked samples were calculated and they ranged from



Chromatogram of a 2.5 min sample showing the peaks of epirubicin and internal standard(I.S.)



Chromatogram of a 1000ng/ml standard with internal standard (I.S.)

Figure (2)-1 : Chromatograms

72.5 - 83.6 (Table (2) - 1). Despite the additional back extraction step, recoveries are comparable to, if not better, than the previous reported methods (33-35).

Calibration curves were plotted and found to be linear for all compounds from 125 ng/ml to 2000 ng/ml with coefficients of regression better than 0.99 (Apendix I P. 131).

The average between-day variability of the assay was 7.6% for epirubicin and 8.2% for 13 - OH (Table (2) - 2).

	RECOVERY (mean±SD) % (n =6)				
	125ng/ml	250ng/ml	500ng/ml	1000ng/ml	2000ng/ml
Epi	73.4±9	77.7±12	80.0±9	72.5±8	81.3±11
13OH	80.0±10	75.0±10	80.2±10	80.0±7	83.6±12

Table (2) - 1 : Recoveries of Epirubicin and epidoxorubicinol (13-OH

Compound	Coeff. of Variation of concentrations % (n = 6)					
	125ng/ml	250ng/ml	500ng/ml	1000ng/ml	2000ng/ml	Mean
Epirubicin	10.3	7.7	7.9	6.2	5.9	7.6
13 - OH	12.4	9.8	8.4	5.1	5.3	8.2

Table (2) - 2 : Between - Day variability of the assay

G. Conclusion

A rapid and economical method of extraction and assay was developed. The sensitivity of the assay was comparable to methods reported earlier. The recovery is satisfactory, taking into consideration the fact that two extraction steps were employed in the procedure. The accuracy and reproducibility are also satisfactory as seen in the calibration curves and the between-day variability, though there is the necessity of a daily run of standard samples when analysing samples with unknown concentrations.

In conclusion, this assay is suitable to perform pharmacokinetic studies of both the parent drug and the metabolite in animal models as well as human subjects.

CHAPTER III : DISPOSITION OF EPIRUBICIN IN PATIENTS WITH HEPATIC CARCINOMA

1. INTRODUCTION

Epirubicin, a stereoisomer of doxorubicin, has been shown in preliminary studies to be effective in treating hepatic carcinoma (9, 16, 17). However, using the conventional routes of administration, efficacy is limited by systemic toxicity. Various experimental drug delivery systems, such as microspheres, liposomes and emulsions, have been described to provide means of targeting tumours with cytotoxic drugs (26, 27, 28). However, one method that apparently has not been explored is the direct mixing of an aqueous solution of a drug with an oil. Lipiodol, a lipid contrast medium has been shown to be taken up preferentially by hepatoma cells and is retained for a long period of time after injection into the hepatic artery (10, 11). This unique property has been exploited by incorporating bleomycin (29), mitomycin-C and adriamycin (30) to Lipiodol for targeting purposes. It is therefore suitable for use as the oily phase in the present study.

2. METHODS

2.1 Patients

Fourteen male patients (age between 34 and 69 years old of Chinese origin), with histologically proven hepatoma or hepatomegaly with a-fetoprotein level above 500 ug/L, were recruited for this study. They had not previously been treated with chemotherapy or radiotherapy and had no serious concurrent illnesses. Eight of these patients were given epirubicin-Lipiodol complex of whom three received more than one course of treatment (the second course at a higher dose) and three patients received only intrahepatic plain epirubicin for comparison. Three other patients on 3 separate occasions received intravenous and intrahepatic-arterial plain epirubicin, and intrahepatic-arterial injection of Lipiodol-drug complex (Table (3)-1). The Lipiodol-epirubicin complex was prepared aseptically by dissolving the prescribed dose of epirubicin with 2 ml of water for injection, and adding to 5 ml of Lipiodol.

TABLE (3) - 1 Demographic Data of patients with liver cancer

Patient	Sex	Age (yr)	Weight (kg)	Body Surface M 2	IA Lipiodol-epirubicin 1st dose (mg)	IA Lipiodol-epirubicin 2nd dose (mg)	Plain epirubicin IA (mg)	Plain epirubicin IV (mg)
LKL	m	57	53	1.60	80	110	-	-
TH	m	69	59	1.71	85	120	-	-
FCN	m	69	70	1.86	95	130	-	-
LYL	m	63	50	1.50	75	-	-	-
CYF	m	34	74	1.98	99	-	-	-
WSK	m	37	53	1.60	80	-	-	-
YKH	m	34	59	1.70	85	-	-	-
CMT	m	37	53	1.60	80	-	-	-
TS	m	68	46	1.40	-	-	70	-
LK	m	38	53	1.60	-	-	80	-
YH	m	57	53	1.60	-	-	80	-
LMT	m	54	61	1.67	-	150	150	150
CKW	m	37	50	1.56	-	140	140	140
KCN	m	58	58	1.68	-	150	150	150

The emulsion was formed by mixing thoroughly within the syringe followed by ultra-sonification. The preparation was infused percutaneously into the hepatic artery under X-ray monitoring according to Seldinger's methods (44) immediately after preparation. Patients received 50 mg m^{-2} or 70 mg m^{-2} of epirubicin. Blood samples were drawn before treatment and at 5, 15, and 30 min and at 1, 2, 4, 8, 12, 24, 48 and 72 hr after administration. Serum samples, separated after centrifugation, were stored at -20° before analysis.

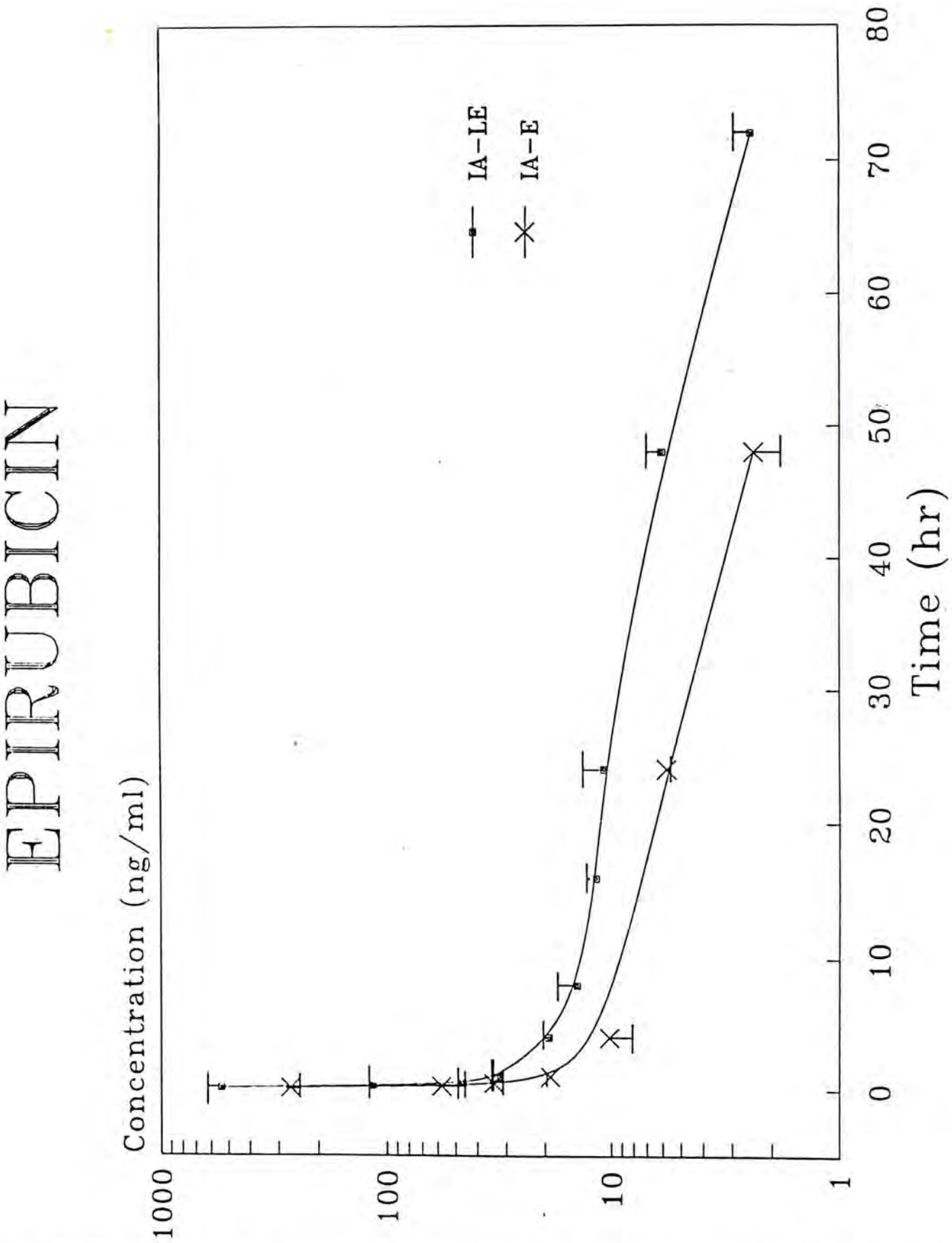
2.2 Assay

The method of assay is as described in Chapter II.

3. RESULTS

Comparison of disposition between IA plain epirubicin and IA Lipiodol-epirubicin

Figure (3)-1 compares the mean serum concentration-time profile of epirubicin in 8 patients who were given intra-arterial (IA) Lipiodol-epirubicin with that from 3 patients given IA plain epirubicin.



Fig(3)-1 : MEAN SERUM CONCENTRATION-TIME PROFILE OF EPIRUBICIN AFTER IA-LE ADMINISTRATION IN 8 PATIENTS AND IA-E ADMINISTRATION IN 3 PATIENTS

Table (3)-2 summarises the mean kinetic parameters. It is apparent that, though the number of patients compared was small, there was a difference in some pharmaco kinetic parameters between these two groups. The AUC_0^∞ is higher in the IA Lipiodol-epirubicin group (IA-LE), while the clearance is faster, and elimination half-life and mean residence time shorter in the IA-plain group (IA-E).

Serum concentration-time profiles of IV, IA epirubicin

The serum-concentration-time curves of epirubicin after intravenous (IV) and intrahepatic-arterial (IA) injection of plain epirubicin, and IA administration of Lipiodol-epirubicin in 3 patients (LMT, CKW and KCN) are shown in Figure (3)-2. The derived pharmacokinetic parameters are summarised in Table (3)-3. It appeared that the relative bioavailability of epirubicin after IA plain epirubicin and IA Lipiodol-epirubicin in the three patients was variable.

Table (3) - 2 Mean (±S.D.) kinetic parameters of epirubicin after intrahepatic-arterial injection of plain epirubicin (IA-E) and Lipiodol epirubicin (IA-LE)

	INTRAHEPATIC-ARTERIAL EPIRUBICIN		
PARAMETERS	IA-E (n = 3)	IA - LE (n = 8)	P <
AUC_0^∞ (ng ml^{-1} h)	0.50 (0.1)	0.84 (0.2)	0.025
Cl (mlh^{-1} Kg^{-1})	3.69 (1.3)	1.84 (0.5)	0.01
Vss (L Kg^{-1})	65.3 (23.1)	48.8 (9.6)	0.25
MRT (h)	17.6 (1.6)	28.0 (8.8)	0.05
$T_{1/2}$ (h)	14.6 (14.6)	22.3 (6.6)	0.05

Figure (3) - 2a Serum concentration-time profile of epirubicin after IV-E, IA-LE and IA-E administration in patient LMT.

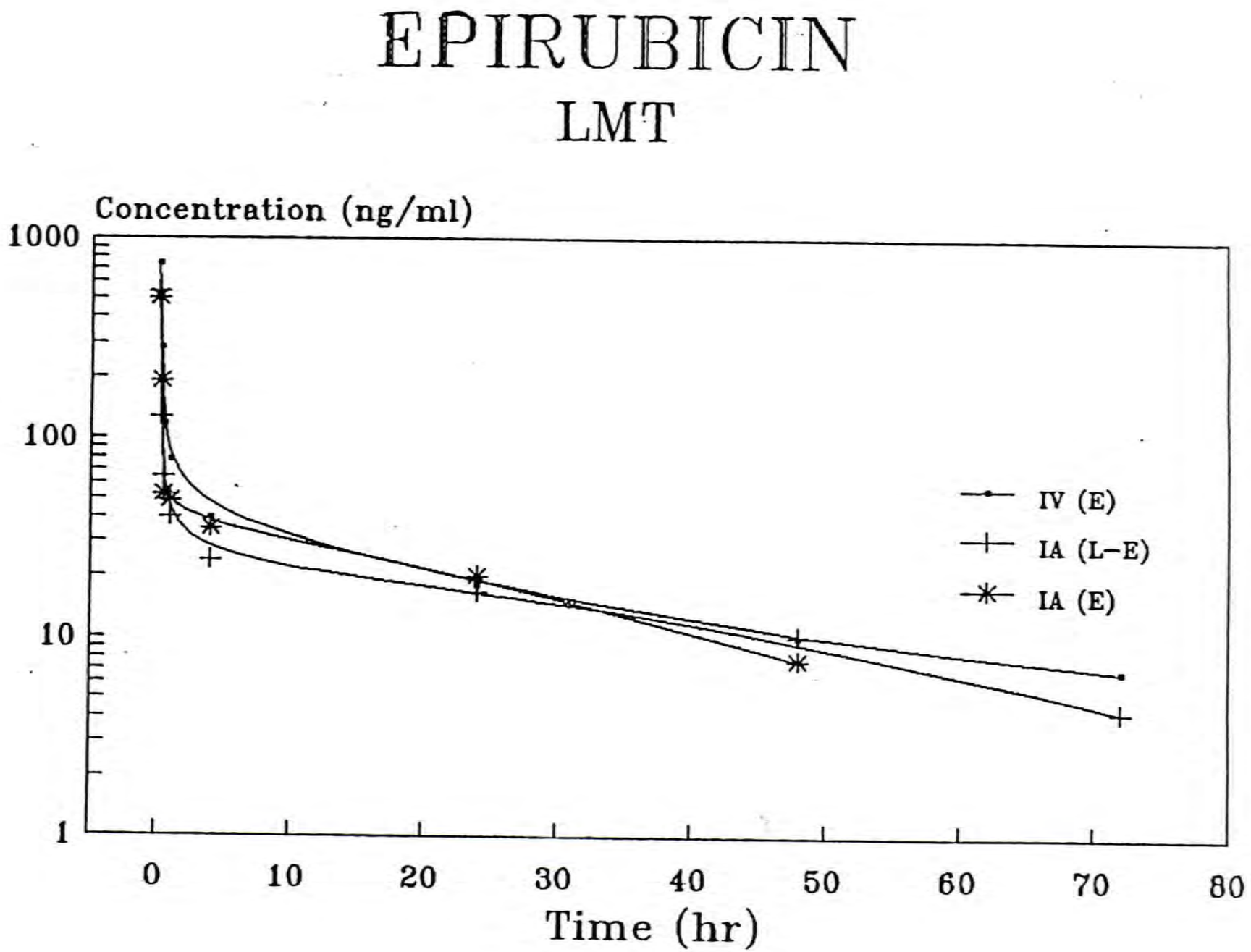


Figure (3) - 2b Serum concentration-time profile of epirubicin after IV-E, IA-LE and IA-E administration in patient CKW.

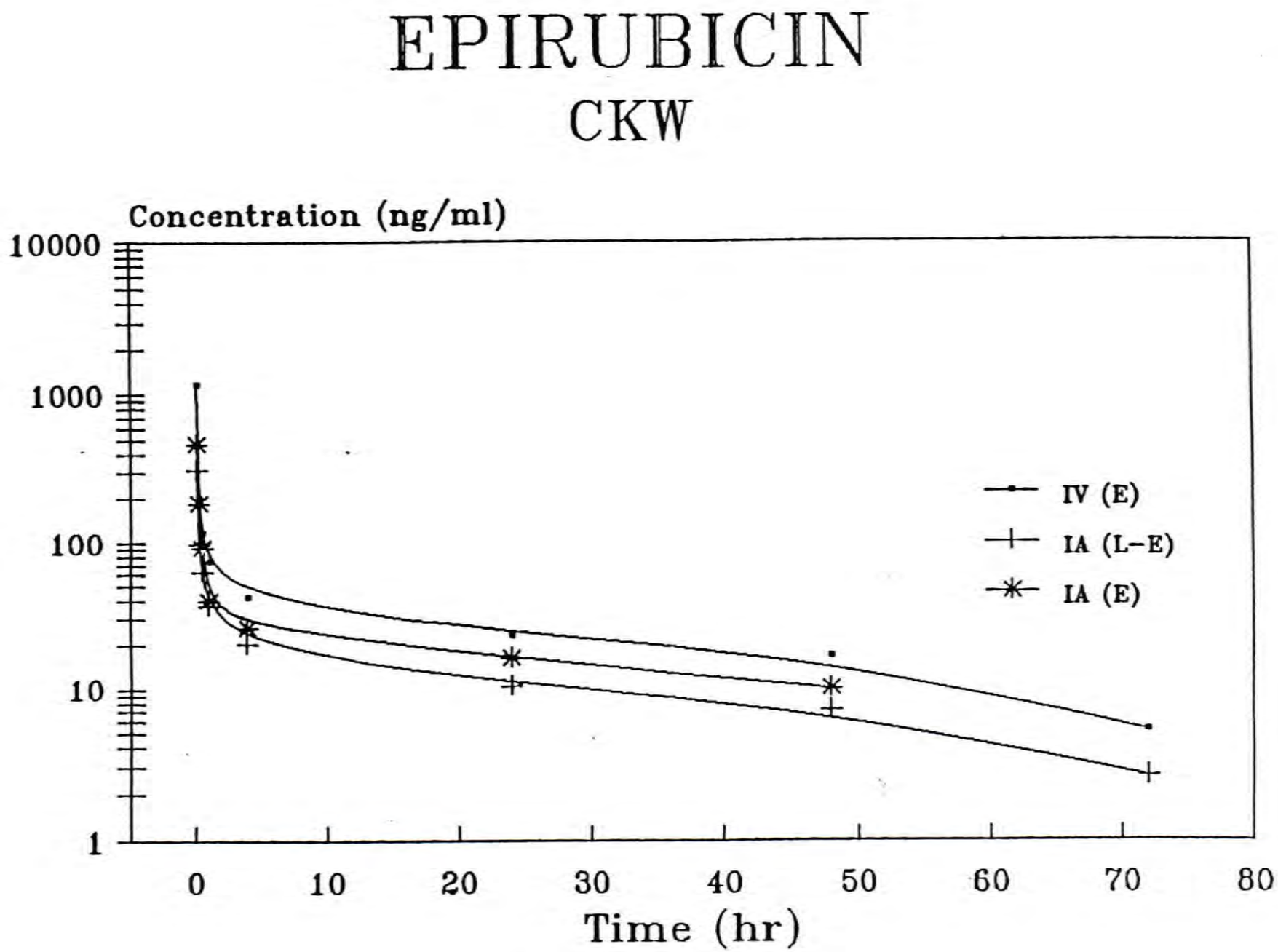


Figure (3) - 2c Serum concentration-time profile of epirubicin after IV-E, IA-LE and IA-E administration in patient KCN.

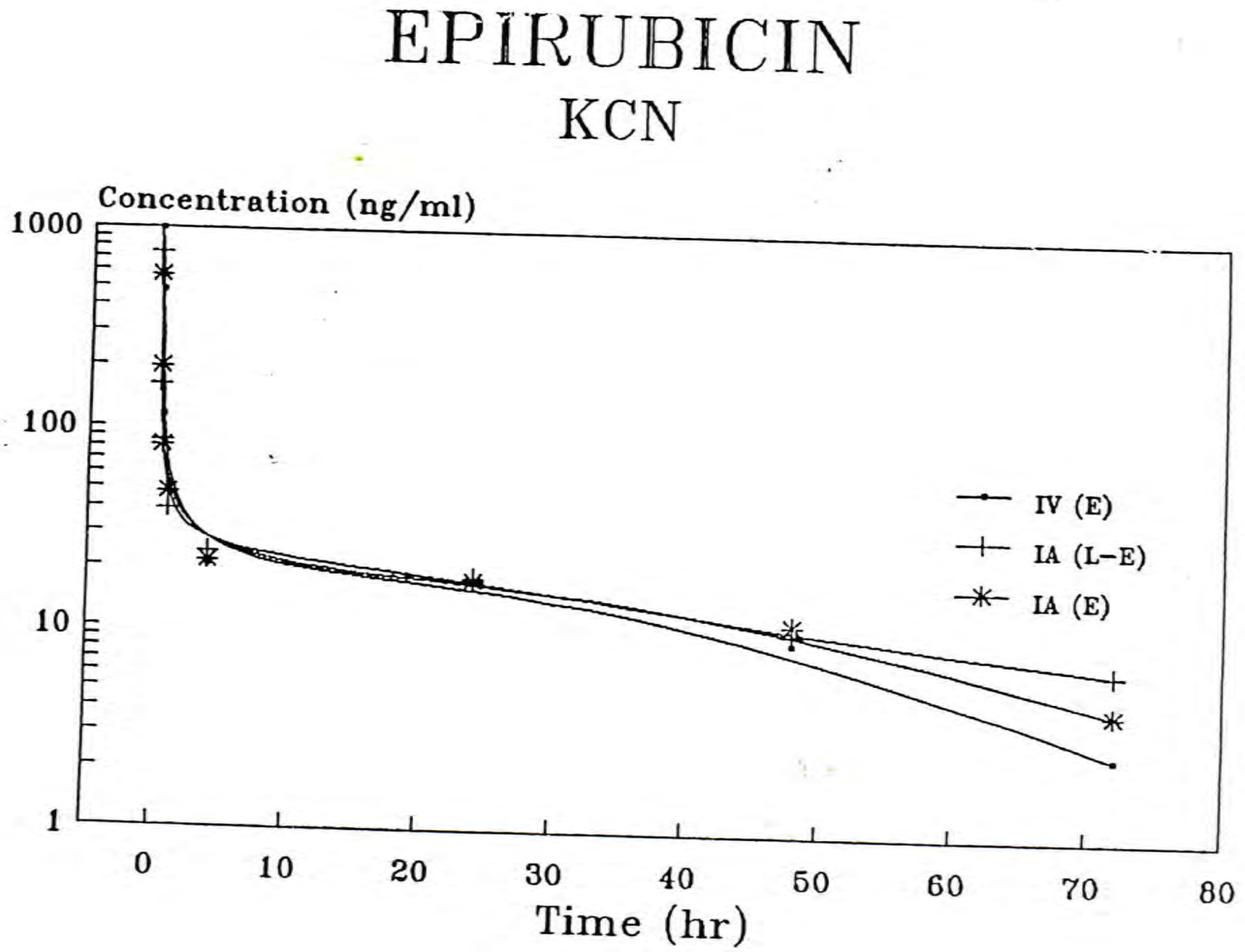
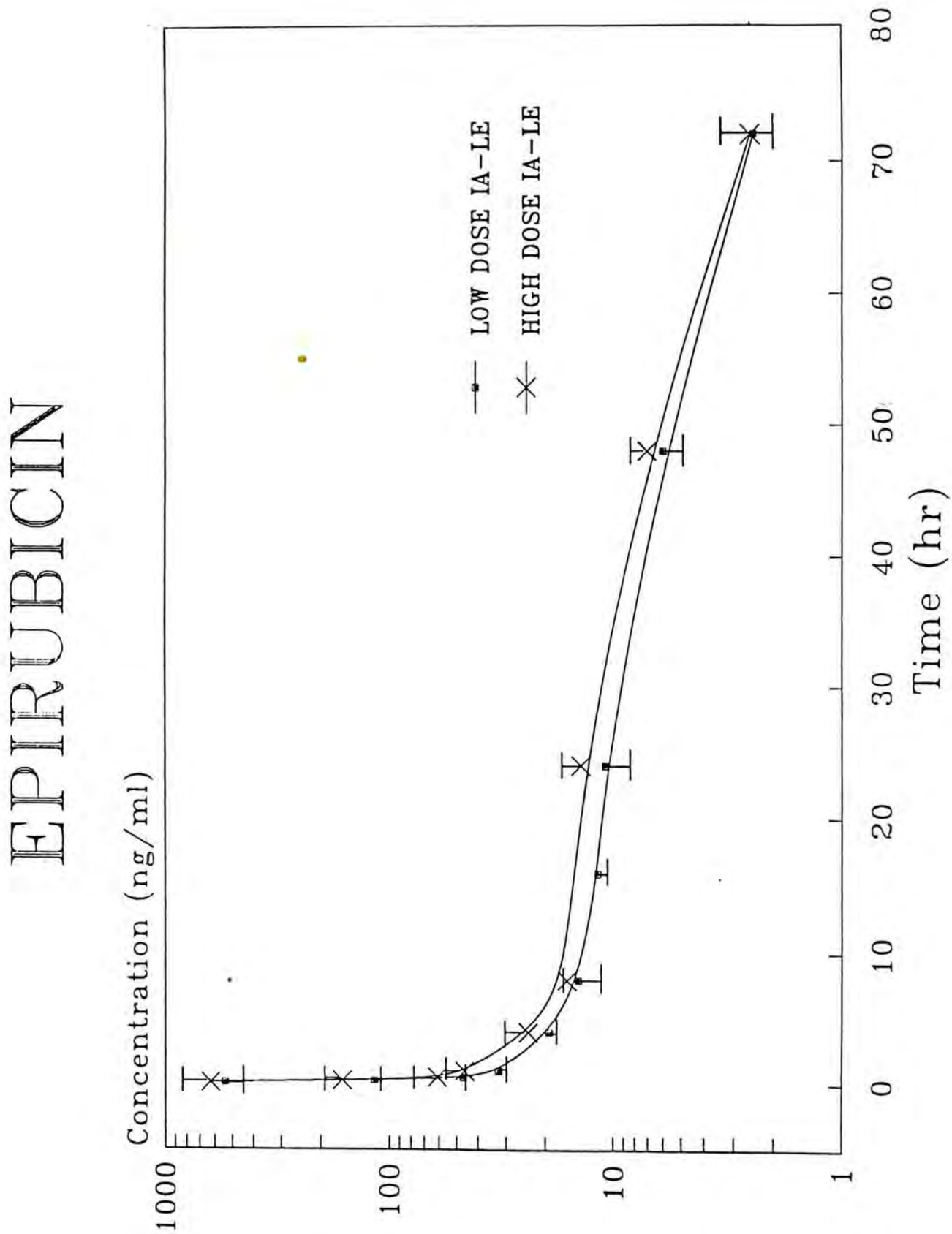


TABLE (3-3) Kinetic parameters of epirubicin in 3 patients after 3 separate doses; intravenous (IV-E), and intrahepatic arterial (IA-E) plain epirubicin, and intrahepatic-arterial (IA-LE) Lipiodol-epirubicin.

PARAMETERS	P A T I E N T S								
	L M T			C K W			K C N		
	IV-E	IA-E	IA-LE	IV-E	IA-E	IV-LE	IV-E	IA-E	IV-LE
AUC_0^∞ ($\mu g\,ml^{-1}\,h^{-1}$)	1.71	1.36	1.30	1.98	1.44	0.90	1.30	1.38	1.56
CL ($ml\,h^{-1}\,Kg^{-1}$)	1.43	1.80	1.89	1.41	1.94	3.10	2.20	1.88	1.63
Vss ($L\,Kg^{-1}$)	46.90	46.50	68.20	42.90	82.90	95.60	53.60	71.00	78.00
MRT (h)	32.70	25.90	36.20	30.40	42.80	30.90	26.70	37.70	46.70
$T_{1/2}$ (h)	27.40	20.20	27.70	23.80	32.90	24.10	22.80	29.50	36.30
Bioavailability	-	0.80	0.76	-	0.73	0.45	-	1.06	1.20



Fig(3)-3 : MEAN SERUM CONCENTRATION-TIME PROFILE OF EPIRUBICIN AFTER HIGH DOSES AND LOW DOSE IA-LE ADMINISTRATION IN 3 PATIENTS

Comparison of disposition between high and low dose of IA Lipiodol-epirubicin

Figure (3)-3 compares the mean serum concentration-time profiles of high and low dose epirubicin after IA Lipiodol-epirubicin in the same 3 patients. There is no apparent difference in the overall pharmacokinetic parameters (Table (3) - 4), the AUC_{0}^{∞}, Vss, MRT and elimination $t_{1/2}$ of epirubicin were bigger in the high dose group, though not statistically significant.

4. DISCUSSION

The present study has shown several important aspects of epirubicin kinetics in patients with liver cancer after IV and IA administration of plain epirubicin and Lipiodol-epirubicin complex. The study, however, was hampered by small patient groups; thus it is difficult to assess the usefulness of the Lipiodol-epirubicin complex to give a clear picture. Patients suffering from cancer in this study were not easy to recruit. Initial trials did include a large number, however, because of the nature of the disease some of them passed away before the next cross-over study.

When intra arterial injection of plain epirubicin (IA-E) was compared with that of Lipiodol-epirubicin (IA-LE) in two different patient groups, there were significant differences in some of the pharmacokinetic parameters (Table (3)-2. Such difference cannot be confidently viewed as an effect of the formulations on epirubicin kinetics for several reasons. Firstly the patient numbers were small. Secondly, a true comparison of epirubicin disposition can only be obtained in crossover experiments as demonstrated by the studies on patients LMT, CKW and KCN (see Table (3)-3). Thirdly the hepatic function of patients with liver cancer might not be stable during the disease period and epirubicin is largely eliminated by liver metabolism. The elimination of epirubicin and its metabolites are impaired in liver dysfunction (45). It was noted that in a cross-over study on the comparative metabolism and pharmacokinetics of epirubicin and adriamycin, a wide variation of metabolic elimination of the epirubicin existed among patients and the elimination of both drugs was significantly impaired in one patient who had hepatic metastases

and percutaneous biliary drainage because of extrahepatic obstruction (45). Furthermore, the Lipiodol-drug complex might not have been homogeneous, the drug release from the complex was not controlled. It is likely that epirubicin might not be trapped in or uptaken by the hepatoma via the Lipiodol.

From the cross-over study in 3 patients who were, on 3 occasions (one month apart), given IV and IA plain epirubicin and IA Lipiodol-epirubicin 70 mg m^{-2}, the relative bioavailability of epirubicin is higher ($\tilde{=}$ 1) in one patient, KCN, (Table (3)-3). It is likely the liver function might be modified by the disease state over a period of 3 months. It is interesting to note that the bioavailability of Lipiodol-epirubicin is lower than epirubicin in the other 2 patients. In all 3 patients, the intravenous plain epirubicin was given as the last dose of the 3 occasions while Lipiodol-epirubicin was given as the first dose. Assuming the liver function of patients LMT and CKW

was not altered throughout the study period, the lower bioavailability of epirubicin after the IA-LE administration suggested some evidence of targeting. The hepatoma might have trapped the Lipiodol-drug complex, thus systemic availability was reduced. The IV data were comparable to published data (46), though patients were of Caucasian origin and with different types of cancer. In a separate cross-over study of three patients who received high and low doses of the Lipiodol-drug complex, slightly larger AUC_0^∞ and longer elimination half life of epirubicin were detected after high dose (Table (3)-4), indicating a dose - related change in the pharmacokinetics of the preparation.

The present study did not give a clear indication whether the Lipiodol-epirubicin complex would give lower blood concentrations of the drug. The reduction in AUC_0^∞ in the cross-over study of three patients might be due to a successful trapping of the drug to Lipiodol which was selectively taken up by the hepatoma or a change in liver metabolic elimination during the course of disease.

TABLE (3)-4 Kinetic parameters of epirubicin in 3 patients after high dose (70 mgm^{-2}) and low dose (50 mgm^{-2}) of intrahepatic-arterial Lipiodol-epirubicin.

PARAMETERS	PATIENTS					
	LKL		TH		FCN	
	High dose	Low dose	High dose	Low dose	High dose	Low dose
AUC_0^∞ (ugml^{-1} h)	0.81	0.71	1.16	0.87	1.19	0.61
Cl (mlh^{-1} Kg^{-1})	2.57	2.12	1.75	1.66	1.57	2.24
Vss (L Kg^{-1})	63.10	48.60	58.20	55.10	51.90	50.6
MRT (h)	24.60	22.90	33.30	33.20	33.10	22.60
$T_{1/2}$ (h)	21.30	23.30	29.10	26.70	24.90	18.60

In conclusion the present study reveals new information to the disposition of epirubicin after intra arterial administration of Lipiodol-epirubicin complex (IA-LE). The IA-LE disposition was comparable with that of intravenous and intra arterial injection of plain epirubicin. Probably it was related to the absence of emulsifying agent in the Lipiodol-epirubicin complex. Further studies are needed to include a better formulation of the complex in order to study the targeting effect toward hepatoma by Lipiodol.

CHAPTER IV : DESIGN OF THE EMULSION FOR INJECTION

PRINCIPLES OF DOSAGE FORM DESIGN

Drugs are almost always given in formulated systems which vary from relatively simple solutions to more complex preparations such as subcutaneous implants. Formulation is achieved by the use of appropriate additives or ingredients, with the help of special technique of compounding if necessary, to provide various pharmaceutical functions and achieving various therapeutic purposes. Hence the principal objective of dosage form design is to achieve a predictable therapeutic response to a drug included in a formulation with reproducible product quality.

There are many factors for consideration in the successful incorporation of a drug into a specific dosage form. These factors can be broadly categorized into three groups:

1. Biopharmaceutical considerations: these include the dosage form, the route of administration, sterility, release of drug from the dosage form, and particle size, etc.

2. Physical and chemical properties of the drug: factors to be considered include the pH, pKa value, stability, compatibility with other ingredients, solubilities in oil and water, etc.

3. Clinical considerations including the acute and chronic toxicity of the formulation, method of administration, and methods of dose monitoring, etc.

For the purposes of this study, we shall concentrate on the biopharmaceutical factors.

BIOPHARMACEUTICAL CONSIDERATIONS

1. Dosage form

Epirubicin is only available in injectable form and therefore any dosage form design using this drug will be based on this preparation.

In this project, the parenteral route is preferred for 2 reasons:

a. an antineoplastic agent is best given intravenously to minimize gastrointestinal upset, increase bioavailability and attain a more predictable blood concentration in a comparatively shorter period of time.

b. depending on the site of administration, an antineoplastic agent can be targeted at certain neoplasm in specific organs by choosing the artery supplying the organ for injection. By limiting the outflow of drug from the specific organ due to accumulation after selective uptake, much higher doses can be given with a lower degree of systemic toxicity.

In many emulsions designed for injection, an oil-soluble medicament is dissolved in a suitable carrier and the mixture is emulsified to form the final preparation. An extra advantage of adopting an emulsion for administration is that the high surface area of dispersed oil globules will enhance the rate of absorption of lipophilic drugs (39). Emulsion for intravenous use must be

of the oil-in-water O/W type (Chapter I) to avoid the formation of emboli whereas water-in-oil (W/O) type emulsions can be administered intramuscularly to produce a depot effect. The choice of emulsifying agents is based mainly on on their efficacy, toxicity, use of final product and the other materials required to be present.

1.1 Choice of oil phase

Konno et al (1 - 8) demonstrated an accumulation of a lymphographic agent, Lipiodol, in liver cancer cells, similar results were also reported by Novell et al (9 - 11). Lipiodol is an iodised poppy-seed oil and usual route of administration is by intramuscular injection and the normal dose in human for diagnostic purposes is 0.15 ml/Kg (42). With this special accumulative effect, together with its low reported toxicity, it was chosen to be the oily phase in the emulsion for this project.

1.2 Choice of Emulsifying Agents

A suitable emulsifying agents for oil - in -

water (O/W) emulsion must be chosen. Emulsifying agents of the non-ionic surfactant type are often used in the preparation of parenteral emulsions due to their low toxicity and irritancy (23), and also a greater degree of compatibility when mixed with oil and water. The only disadvantage is that they tend to be more expensive.

Non-ionic surfactants can exhibit both hydrophilic and hydrophobic properties due to their surface active character. By varying the relative proportions of components, products of different texture can be produced. If the hydrophobic part of the molecule predominates then the compound will have greater solubility in oil, and hence will migrate and concentrate in the oil phase which becomes the continuous phase of an emulsion, producing a water-in - oil (W/O) emulsion. Similarly a surfactant with higher water solubility will give an oil-in-water (O/W) emulsion.

The best type of non-ionic surfactant to use is therefore one with an equal balance of hydrophilic and hydropholic properties. Common examples include lecithin, polysorbates, methylcellulose, gelatin and serum albumin. In this project, non-ionic surfactants - a polysorbate with a sorbitan ester, were chosen for the following reasons:

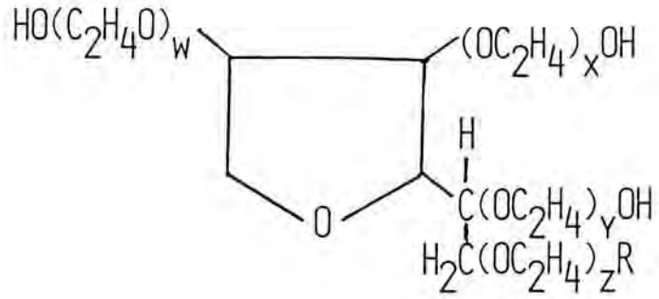
a. compatible with most anionic, cationic and non-ionic materials

b. neutral pH

c. stable to the effects of heat, pH changes, and high concentrations of electrolyte

d. low systemic toxicity

Polysorbates are polyethylene glycol derivatives of sorbitan esters. They have the general formula :

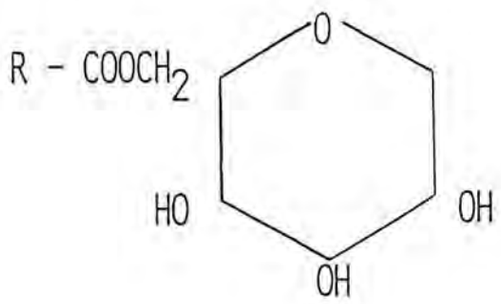


(SUM OF W,X,Y AND Z IS 20;
R IS $(C_{11}H_{23})COO$)

General Formula of Polysorbates

where R represents a fatty acid chain. Variations in the type of fatty acid used and in the number of oxyethylene groups in the polyethylene glycol chains produces a range of products of differing oil and water solubilities.

Sorbitan esters are produced by the esterification of one or more of the hydroxyl groups of sorbitan with either lauric, oleic, palmitic or stearic acid. They have the general formula



General Formula of Sorbitan Esters

where R represents a fatty acid chain.

To increase the stability of an emulsion, polysorbates are generally used in conjunction with the corresponding sorbitan ester to form a complex condensed film at the oil/water interface (39).

For this project, polysorbate - 20 and its corresponding sorbitan ester, sorbitan monolaurate, were chosen to be the main emulsifying agents because of superior solubility in water, and hence a greater tendency to produce an oil - in - water (O/W) emulsion than other analogs (39).

Stability of the emulsion can be enhanced by the addition of stabilizers. One group of commonly used stabilizer in emulsion are the poloxamers which are employed in intravenous fat emulsions and as solubilising agents to maintain clarity in elixirs and syrups. They are a series of non-ionic polyoxyethylene polyoxypropylene copolymers with the general formula

$$HO(C_2H_4)_a - (C_3H_6O)_b - ((C_2H_4O)_c - H$$

where a = c. An additional advantage of including poloxamer into the emulsifying system is : that poloxamer may increase the absorption of fat-soluble substances (39). Thus for the purpose of this project, it might help to facilitate the uptake of lipid-soluble drug molecules into cancer cells and secure a targeting effect.

2. Route of administration

A satisfactory response and a high degree of safety were demonstrated in addition to a targeting effect when antineoplastic agents were injected via the hepatic artery or the hepatic portal vein for the treatment of unresectable hepatocarcinomas (1 - 9). Moreover, this method should produce a much higher concentration of drug in the liver circulation yet a lower degree of systemic toxicity due to lower systemic plasma concentration after accumulation. The hepatic portal vein is therefore chosen to be the site of administration for the emulsion. It is preferred to the hepatic artery due to its more superficial anatomical position in rabbits, which are the major experimental models in this study.

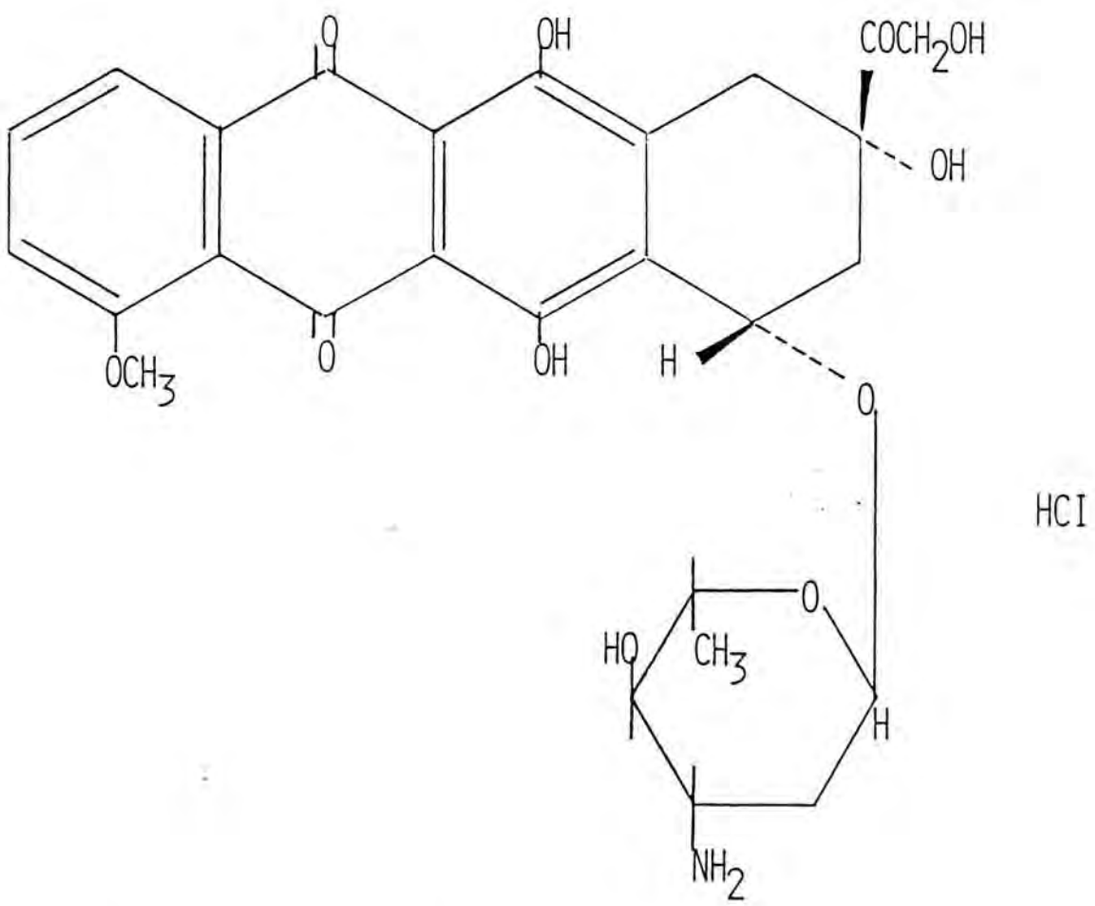
PHYSICAL AND CHEMICAL PROPERTIES OF THE DRUG

In this project, epirubicin in the form of its HCl salt is used during the entire study due to the unavailability of its free base.

1. Chemistry of epirubicin

Chemical data of epirubicin HCl are as follows (15) :

Structural formula :



EPIRUBICIN HYDROCHLORIDE

Chemical formula	:	$C_{27}H_{29}NO_{11}HCl$
Molecular weight	:	579.98
Physical properties	:	red-orange, practically odourless, crystalline powder.
Solubility	:	soluble in water and DMSO, slightly soluble in absolute ethanol, practically insoluble in non - polar solvents.
Melting point	:	177°C with decomposition
pH	:	pH of a 0.5% aqueous solution is 4.0 - 5.5
pKa	:	pKa of the NH_3^+ group, determined by aqueous titration at 25°C with 0.1N NaOH is 7.7

2. Stability and compatibility of the formulated emulsion compared to that of an aqueous solution.

According to the data supplied by the original manufacturer, a reconstituted solution retains up to 98% of its potency in 24 hours when stored at room temperature. It is also recommended that aqueous solution may be stored for up to 48 hours if refrigerated after reconstitution.

It is recommended that epirubicin should not be mixed with heparin due to incompatibility due to possible chemical reactions, and it should not be mixed with other antineoplastic agents in the same intravenous fluid or the same syringe.

These factors were thoroughly considered in the development of the formula for epirubicin emulsion and careful observations was taken every time after preparing the emulsion to check for possible precipitation.

CLINICAL CONSIDERATIONS

One of the major concerns in developing a dosage form is its toxicity - both acute and chronic. Since this project involves a cancer chemotherapeutic agent, chronic toxicity is probably of less significance because the drug is usually given as bolus doses in short periods of time. Hence emphasis should be placed on detection of acute toxicity. A thorough study of this is described in Chapter V.

The actual method of administration and dose monitoring in human subjects should be determined in conjunction with clinicians involved in future clinical trials.

THE FORMULA OF THE EMULSION

The ingredients of the emulsion were discussed in earlier parts of this chapter and this section deals mainly with the determination of the percentage of each ingredient in the formulation.

1. Percentage of oil

Pharmaceutically, it is not easy to stabilize an emulsion containing less than 20% disperse phase since creaming would occur readily (36). A higher disperse phase concentration would result in a hindrance of movement of the droplets and hence a reduction in rate of creaming. On the other hand, although it is theoretically possible to include as much as 74% of an internal phase, it is usually found that at about 60% concentration phase inversion occurs (36, 37). The next point of consideration is, of course, the degree of toxicity offered by this range of percentage of oil and this is revealed by the studies on acute toxicity described in Chapter V. From this, a much higher LD-50 was obtained for the emulsion with 25% of oil incorporated. Together with the consideration that a higher percentage of oil might necessitate a higher percentage of emulsifying agents, hence a higher possibility of toxic effects, we therefore set the level of oil at 25% (V/V).

2. Percentage of emulsifying agents

When a polysorbate and its corresponding sorbitan ester are used as the emulsifying system in an

emulsion, the recommended total percentage normally ranges from 5% - 12% (23, 29). Taking into consideration possible systemic toxicity, 5% was chosen as the concentration for the emulsion; this would be increased if stability proved to be a major problem.

The proportions of polysorbate - 20 and sorbitan monolaurate in this 5% (W/V) of emulsification system is calculated using the Hydrophile - Lipophile Balance (HLB) method (40).

Calculation :

Required HLB value of oil in an O/W emulsion = 12

HLB value of polysorbate 20 = 16.7

HLB value of sorbitan monolaurate = 8.6 (41)

Let X = % of sorbitan monolaurate in the mixture

where 100-x = % of polysorbate 20 in the mixture.

$$\text{Contribution from sorbitan monolaurate} = \frac{x}{100} \times 8.6$$

$$\text{Contribution from polysorbate 20} = \frac{(100 - x)}{100} \times 16.7$$

Since the total required HLB is 12

$$\left(\frac{x}{100} 8.6\right) + \left[\frac{(100 - x)}{100} \times 16.7\right] = 12$$

$$x = 58\%$$

Therefore sorbitan monolaurate will occupy 58% (W/W) and polysorbate-20 will occupy 42% (W/W) of the total 5% of the mixed emulsifying system in the emulsion.

As for poloxamer, a concentration of 2.2% (W/V) had been used in studies as the only emulsifier with no toxicity reported (39). In this project, the concentration is set at 2% as its role in this case is an emulsion stabilizer.

With the above considerations, the initial formula of the Lipiodol - epirubicin emulsion is as follows:

Lipiodol		25% V/V
Polysorbate 20	}	5% W/V
Sorbitan monolaurate	}	
Poloxamer		2% W/V
Epirubicin		10 mg
Water for injection to		5 ml

THE METHOD OF PREPARATION OF THE EMULSION

The preparation was carried out in a multiple-dose vial of 10 ml capacity.

Procedure:

1. Lipiodol was added into the vial.

2. Suitable quantities of polysorbate 20, sorbitan monolaurate and poloxamer were weighed directly into the vial.

3. About 3 ml of water for injection was used to reconstitute the epirubicin, the mixture was then shaken until completely dissolved.

4. The epirubicin solution was added to the Lipiodol - emulsifiers mixture.

5. Water for injection was added to adjust to the final volume.

6. The emulsion was placed in an ultrasonicator (42, 43) to facilitate complete dissolution of the solids and breaking down of the oil phase into smaller globules.

7. The whole product was filtered using a 0.2 um disposable sterile membrane filter, and collected into a pre-sterilized multiple-dose vial.

BIOPHARMACEUTICAL STUDIES ON THE FORMULATED EMULSION

1. RELEASE OF DRUGS FROM THE FORMULATED EMULSION

The rate of release depends mainly upon the oil/water partition coefficient of the drug and its rate of diffusion across the oil phase. The release of epirubicin when it is incorporated in an emulsion can be demonstrated using a diffusion

cell mounted on a cell-driving console and maintaining the temperature at 37°C (human body temperature) using a warm water circulator.

1.1 Method and Materials

A two-compartment diffusion cell (Advanced Pharmaceutical Systems, Inc., New Jersey, U.S.A.) was mounted on a cell-driving console. One cell (referred to as cell A) was filled with 2.5 ml of an isotonic pH 7.4 phosphate buffer while the other cell (cell B) was filled with 1 ml of epirubicin emulsion (concentration: 2 mg/ml) plus 1.5 ml of the same buffer as cell A. The 2 cells were separated by a molecularporous membrane (Molecular Weight Cut-off MWCO 12,000 - 14,000) (Spectrum Medical Industries Inc., Calif., USA). Warm water at about 37°C was circulated around the cells by a water-circulator.

The magnetic stirrer was turned on and samples of 20 ul were taken at time 0 (control), and 30 minute intervals thereafter up to 6 hours from both cells A and B.

The same procedure was repeated using epirubicin aqueous solution (concentration : 2 mg/ml) in place of epirubicin emulsion in cell B. Extraction and analysis using HPLC were carried out according to the method described in Chapter II. The whole experiment was repeated three times.

1.2 Results and discussion

The results of HPLC analysis are shown in Table (4) - 1 (Appendix II). The comparison between the release of drug from the formulated emulsion and plain solution is shown in Fig. (4) - 1 which shows the decrease in concentration of epirubicin from the original preparations. The rate of diffusion of drug across the membrane, hence the increase in concentration of epirubicin in the buffer solutions in each case is demonstrated in Fig. (4) - 2.

From the graphs it is apparent that the diffusion ability of epirubicin is not impaired after its incorporation into the formulated emulsion although there is a one-hour delay before any epirubicin from emulsion enters the buffer. Yet from Fig. (4) - 2, it is interesting to note that the rate of

Fig.(4)-1: Change of Conc. with Time in the Epirubicin Emulsion & Solution

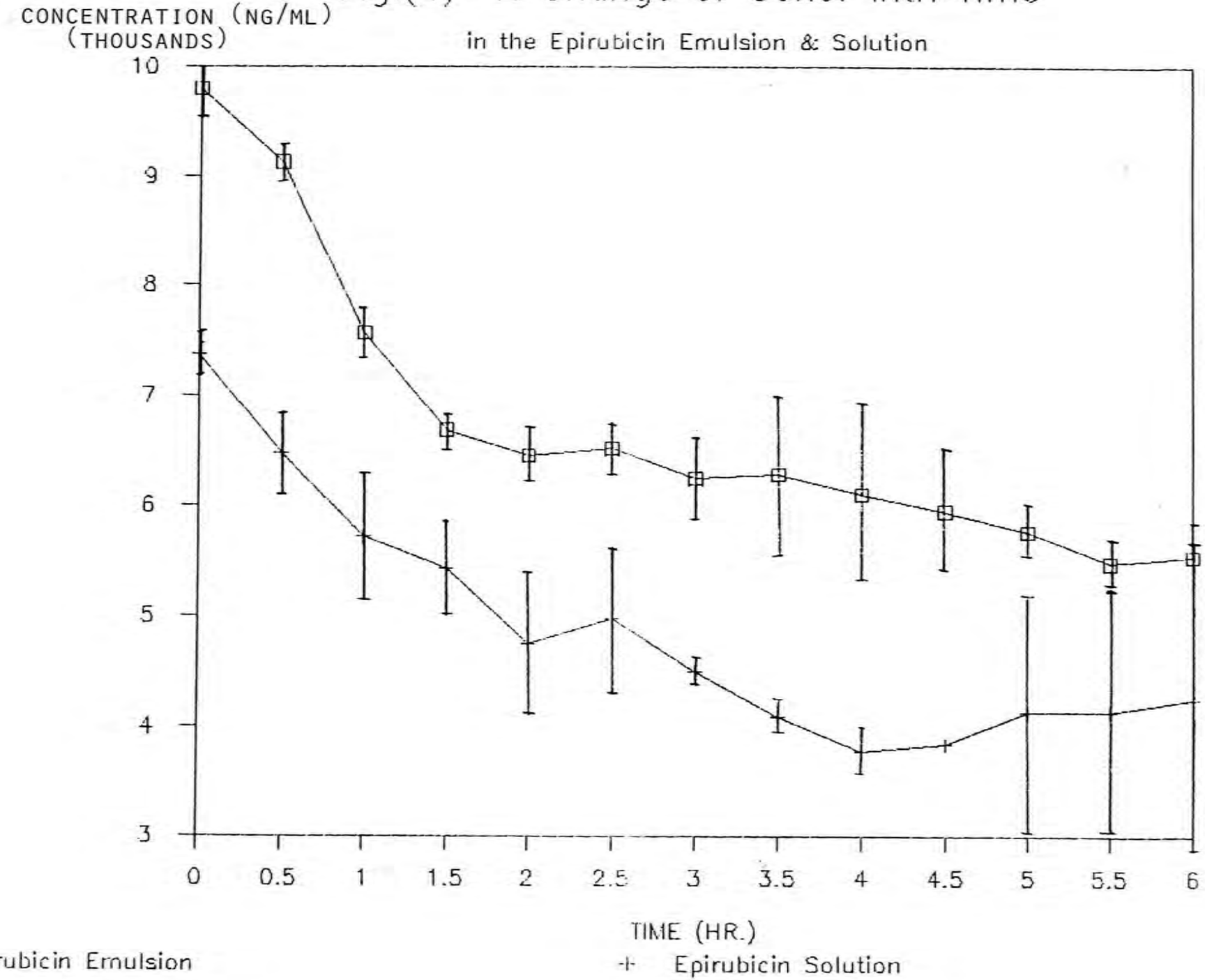
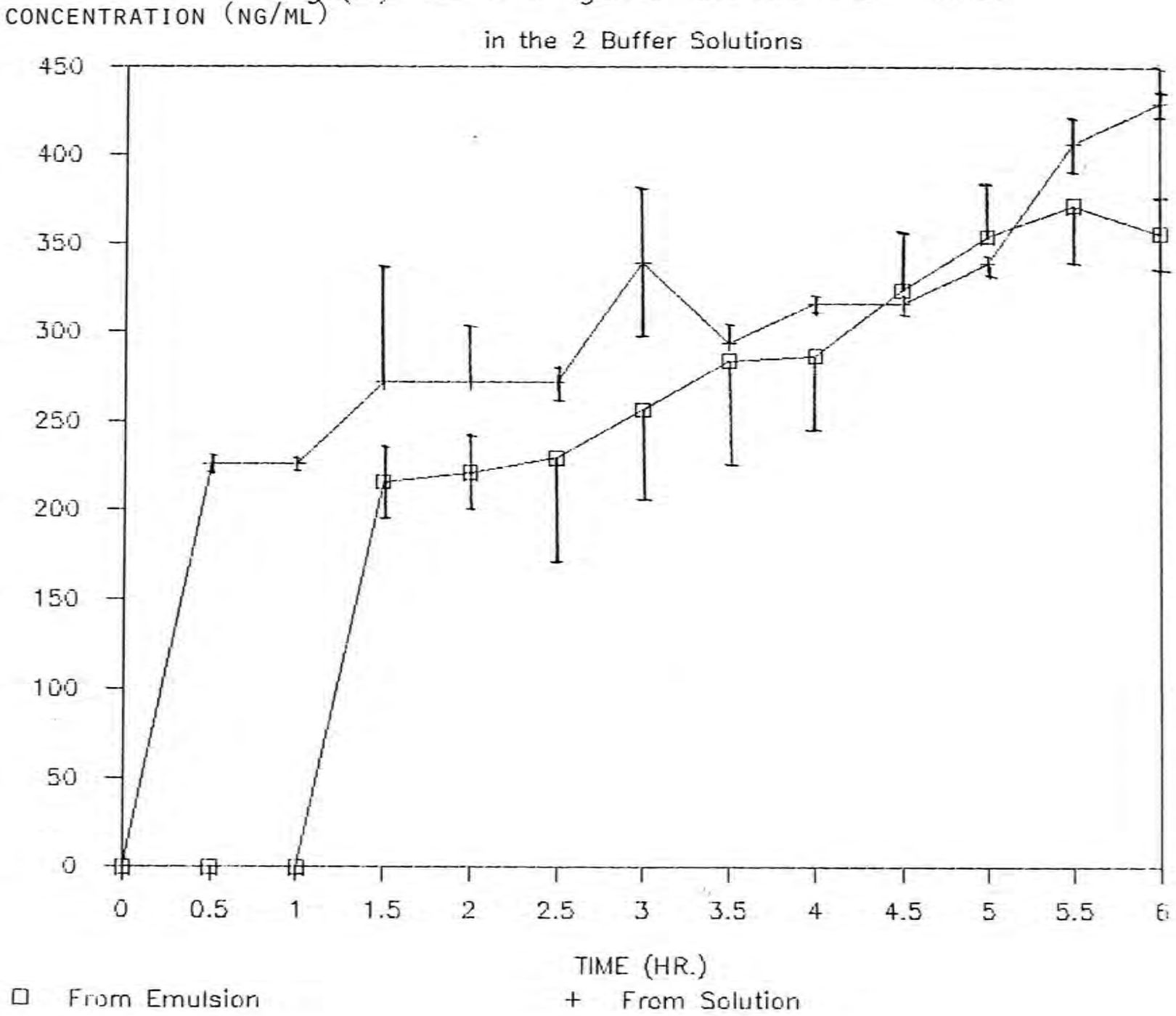


Fig.(4)-2: Change of Conc. with Time in the 2 Buffer Solutions



diffusion of epirubicin from the emulsion is somewhat slowed down, though not to a significant level. It should also be noted that after 6 hours, the final concentration attained in buffer is somewhat higher for epirubicin solution than emulsion. Hence there is a possibility that some drug molecules are trapped in oil globules resulting in a lower diffusion rate and diffusion ability over the 6 - hour period.

2. GLOBULE SIZE OF OIL IN AN OIL-IN-WATER (O/W) EMULSION.

The globules of an O/W emulsion for intravenous or intra-arterial use must be kept smaller than 4 um to avoid the formation of emboli (23). Traditionally, this can be achieved through a careful selection of emulsifiers and emulsion stabilizers.

In this project, a special method was developed to break the oil phase into smaller globules, hence producing a more stable emulsion and reducing the risk of emboli on administration.

2.1 Method

The finished product was drawn into a 10 ml syringe. A 0.2 um disposable sterile membrane filter (S&S, Germany) was then attached to the syringe. The emulsion was then slowly filtered through the membrane and the filtrate was collected in a sterilized injection vial. Examination of the particle size was done under a microscope with the help of a graticule.

2.2 Results and Discussion

Microscopic examination of the filtrate revealed a majority (about 80%) of the dispersed oil globules was in the range of 1-3 um in diameter. Although safety for administration is not absolutely guaranteed, the risk of causing emboli should be considerably less than a simple mixing of aqueous phase and oily phase by ultrasonification. Moreover, the method provides a convenient and economical way to

sterilize the preparation at the same time as oil globules are reduced in size because it is recommended by the Pharmaceutical Codex that sterilization by filtration must be done with filter of the pore-size range 0.2 - 0.22 um (36).

3. STABILITY OF THE FORMULATED EMULSION

According to the manufacturer's recommendation (15), epirubicin injection, after reconstitution, retains up to 98% of its potency for 24 hours at room temperature, and for 48 hours in a refrigerator (4 - 10°C). The present project incorporates epirubicin into an emulsion, and it is interesting to find out whether the presence of other ingredients in the formulated emulsion would influence the stability of epirubicin.

3.1 Method

Epirubicin emulsion was prepared and samples were taken right after preparation and 24, 48, 72 and 96 hours afterwards, the preparation was

refrigerated at 2 - 4 C throughout, and the study was repeated 3 times . Extraction and analysis by HPLC were performed as described in Chapter II.

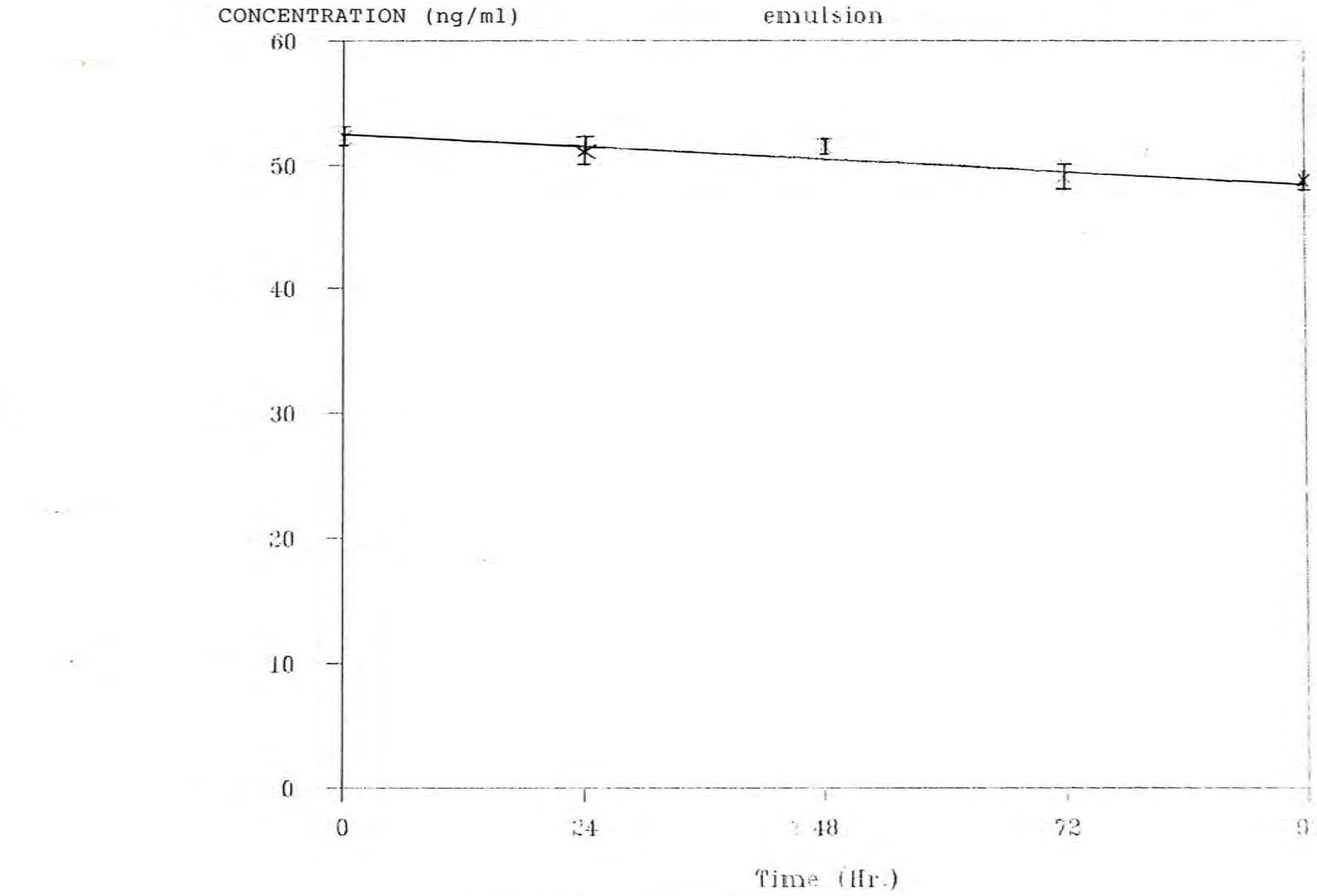
3.2 Results and discussion

The results of HPLC analysis are shown in Table (4) - 2, and Figure (4) - 3 demonstrates the stability of the drug when kept at 0 - 4°C after reconstitution. Results show that after epirubicin is incorporated in an emulsion, its stability is apparently unaffected. As a matter of fact, it is quite reasonable to say that it is just as stable as a solution if it is kept refrigerated right after reconstitution.

Time (hr.)	Emulsion A	Emulsion B	Emulsion C	Mean S.D.
0	53.1	52.1	51.55	52.25±0.78
24	52.4	50.3	50.8	51.17±1.10
48	50.8	52.3	51.4	51.5±0.62
72	49.2	47.9	49.7	49.1±0.93
96	49.6	48.4	48.4	48.8±0.69

Table (4) - 2 : Concentration (mg/ml)of Epirubicin in the formulated emulsion at 0 - 4°C

Fig(4)-3: Stability of Epirubicin in an emulsion



4. ACCELERATED STABILITY TESTS

The stability of refrigerated epirubicin emulsion has been established. It will also be of interest to know whether other adverse storage conditions, of which temperature being the most significant one, would induce an accelerated disintegration of the emulsion.

4.1 Method

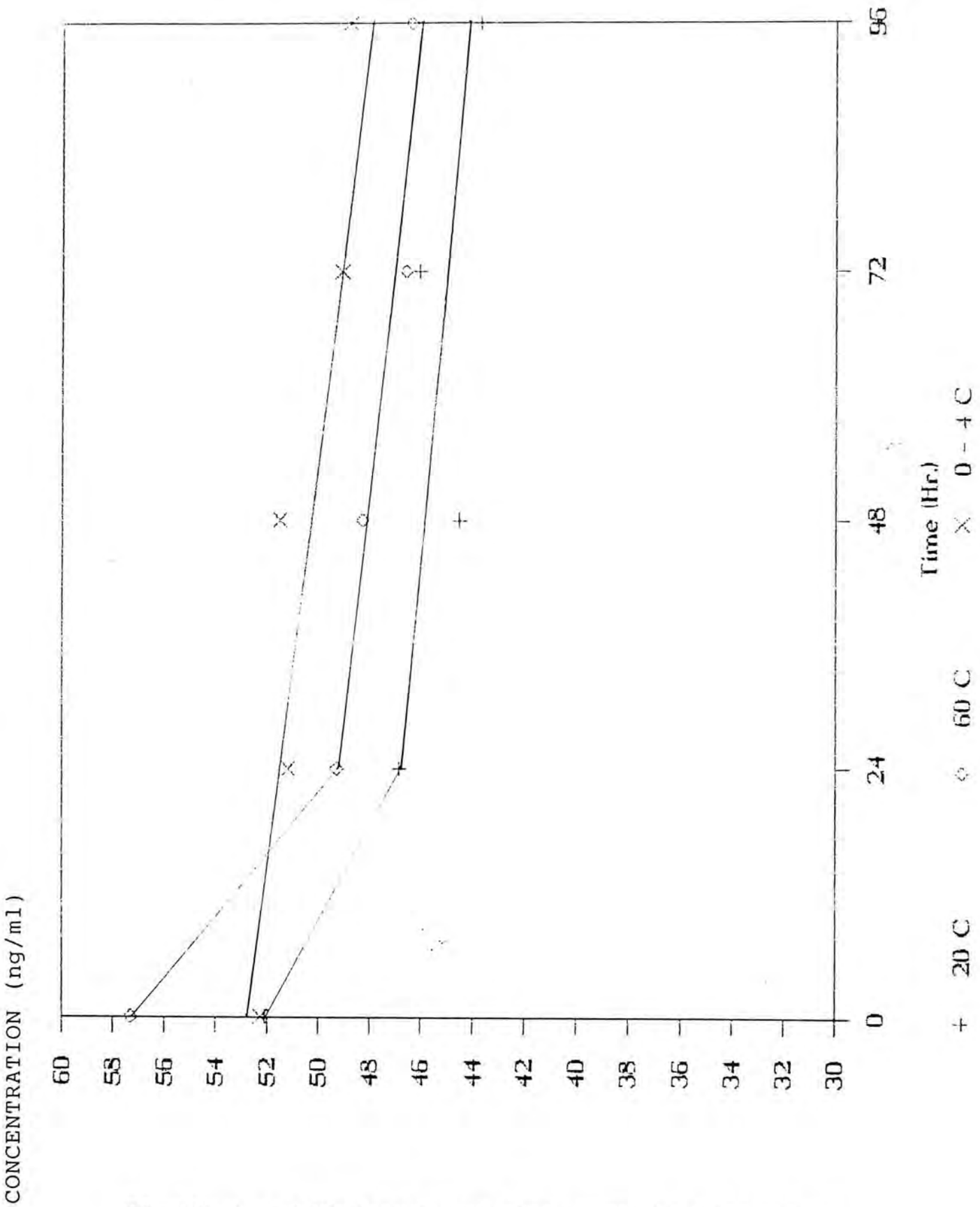
The formulated emulsion was prepared and it was then placed in an incubator, temperature being set at 20°C (room temperature). Samples of 20 ul were drawn at time 0, then 24, 48, 72 and 96 hours afterwards. Extraction and analysis were performed according to the procedure discribed in Chapter II. The same was repeated with temperature set at 60°C.

4.2 Results and Discussion

Results of the tests are as listed in Table (4) - 3 and Table (4) - 4. Figure (4) - 4 shows when compared with the stability test done previously, the disintegration of epirubicin emulsion appears to be enhanced with increassing temperature. Therefore it is important to refrigerate the emulsion after preparation and 48 hour appears to be a reasonable shelf-life for the formulation if kept refrigerated.

Time (hr.)	Emulsion A	Emulsion B	Emulsion C	Mean ± S.D.
0	51.95	52.05	52.2	52.1 ± 0.13
24	47.2	47.4	46.07	46.85 ± 0.72
48	46.9	47.1	39.5	44.5 ± 4.33
72	45.4	47.2	45.6	46.06 ± 0.99
96	44.3	42.9	43.8	43.7 ± 0.71

Table (4) - 3 Concentration (ng/ml) of Epirubicin Emulsion at 20°C



Fig(4)-4 : COMPARISON OF STABILITY OF EPIRUBICIN EMULSION MAINTAINED AT DIFFERENT TEMPERATURES

Time (hr.)	Emulsion A	Emulsion B	Emulsion C	Mean ±S.D.
0	57.3	56.9	57.73	57.3 ± 0.42
24	50.3	47.2	50.4	49.3 ± 1.82
48	51.1	45.3	48.4	48.3 ± 2.90
72	48.3	45.1	46.4	46.6 ± 1.61
96	47.4	45.3	46.7	46.4 ± 1.07

Table (4) - 4 Concentration (ng/ml) of Epirubicin Emulsion at 60°C

5. STERILITY OF THE FORMULATED EMULSION

As the emulsion will be injected either intravenously or directly into the liver via the hepatic portal vein, it must be maintained in a sterile state before and after the incorporation of epirubicin. Traditionally an emulsion for injection is sterilized by either one of the following methods : ultra - filtration, dry / wet heat sterilization, irradiation or simply prepared using aseptic techniques.

5.1 Method

Every time either a plain vehicle or a medicated emulsion was prepared, culture was done on nutrient agar and incubated at 37° C for 48 hours.

5.2 Results and Discussion

No microbial growth has been demonstrtated so far in any of the preparations. The absence of growth demonstrates the efficacy of the process of microfiltration by 0.2 um filter membrane. Lack of microbial growth may also be due to the effect of epirubicin itself as it is a derivative of the anthracycline group of antibiotics.

These findings enable us to avoid drastic sterilization procedures such as autoclaving. Nevertheless, the fact remains that it is important for us to perform the admixture under a laminar flow hood and use glassware and utensils sterilized before use.

To conclude, the preparation should be safe in terms of sterility for intravenous administration into a peripheral vein or directly via the hepatic - portal vein.

6. DEMONSTRATION OF HEPATIC UPTAKE AND RETENTION OF THE EMULSION

6.1 Method

The emulsion was prepared according to the method and formula described above. A rabbit was anesthesized by intravenous administration of pentobarbitone.

A catheter was inserted into the hepatic portal vein after an incision was made in the thoracic - abdominal region. An appropriate dose of the emulsion was injected slowly through the catheter over 1-2 minutes.

X - ray films were taken at time 0 which was the control, right after administration was completed, and at 1, 2, 5 and 10 minutes afterwards.

The same procedure was repeated with epirubicin - Lipiodol mixture (i.e. without the emulsifier system).

6.2 Results and Discussion

The X - ray films taken using epirubicin emulsion are shown in Figure (4) - 5 to Figure (4) - 10 while those using epirubicin - Lipiodol mixture in Figure (4) - 11 to Figure (4) - 16.

An obvious retention effect was observed as a considerable amount of the emulsion can still be seen in the liver lobes 10 minutes after administration. A selective uptake effect is possible because the image of the blood vessels is fading while the surrounding areas are becoming opaque as can be seen from Figures (4) - 4 and (4) - 5. This appears to be due to a facilitated entry of the oil into the hepatocytes after formulation into an emulsion, and drugs trapped in the oil globules could therefore be delivered into cells more efficiently. As for epirubicin - Lipiodol mixture, a rapid disappearance is observed, indicating a rapid drainage of the fluid from the liver i.e. no retention effect was obtained.

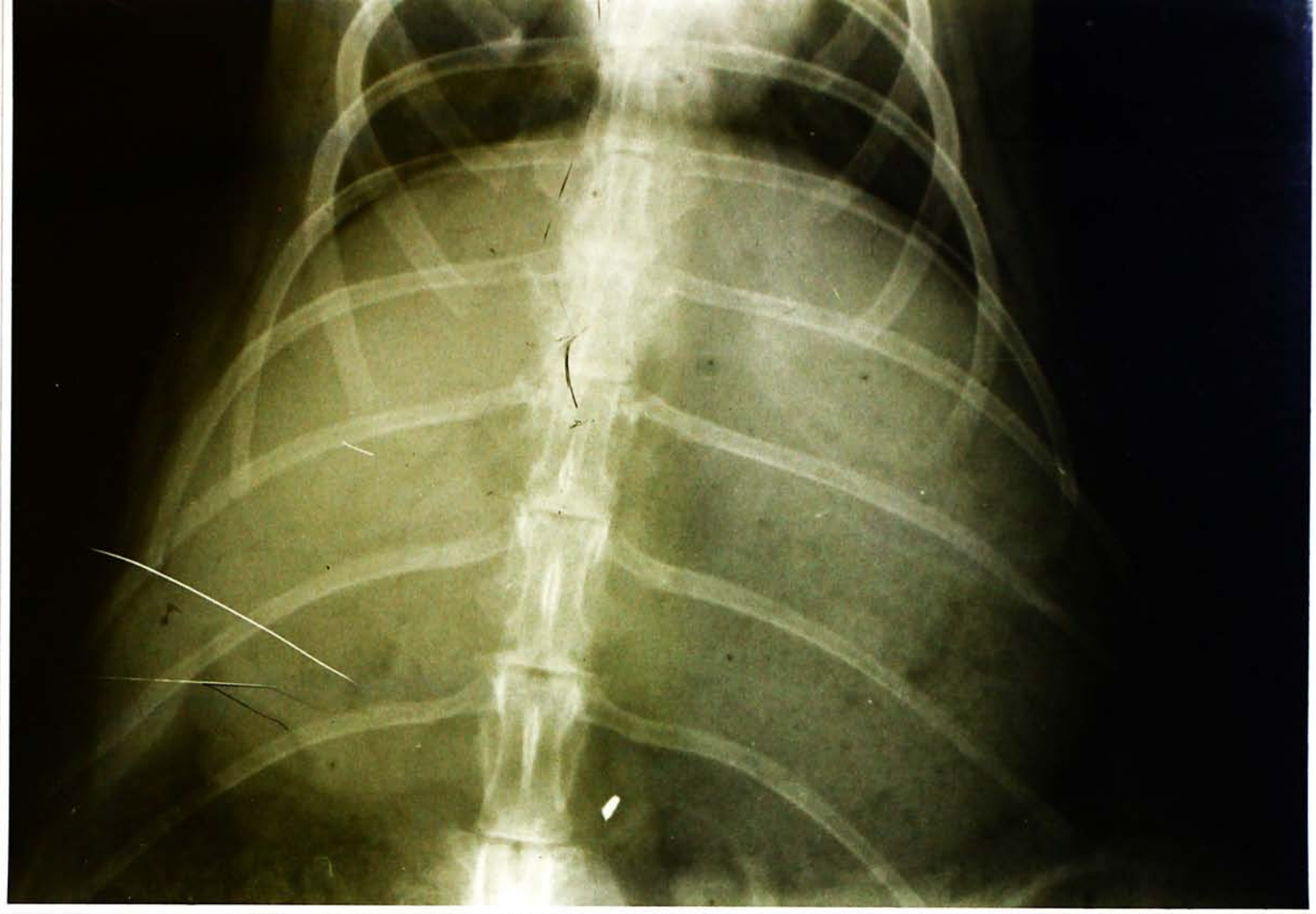


Fig. (4) - 5 : Control before injection of emulsion

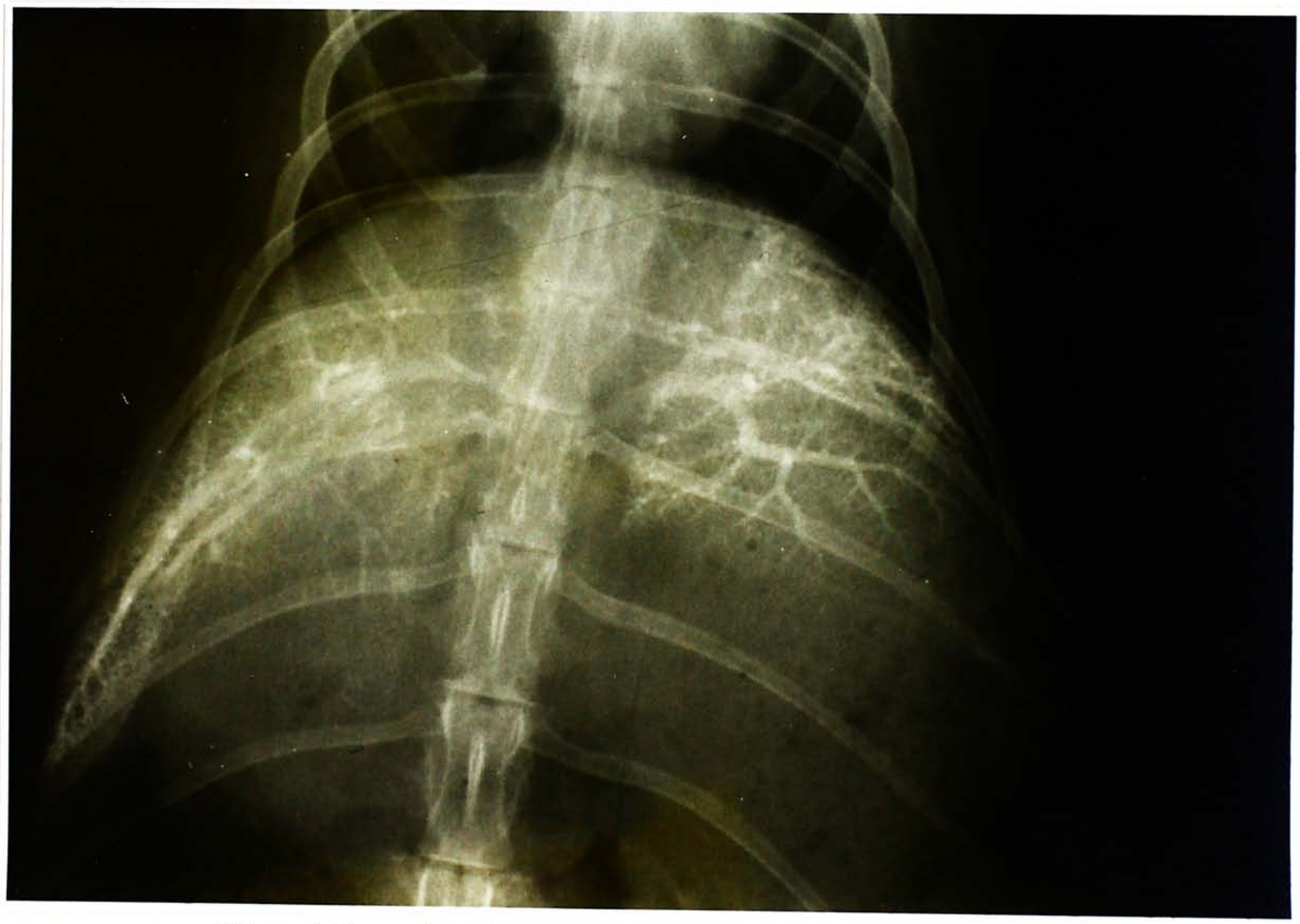


Fig. (4) - 6 : Immediately after injection of emulsion

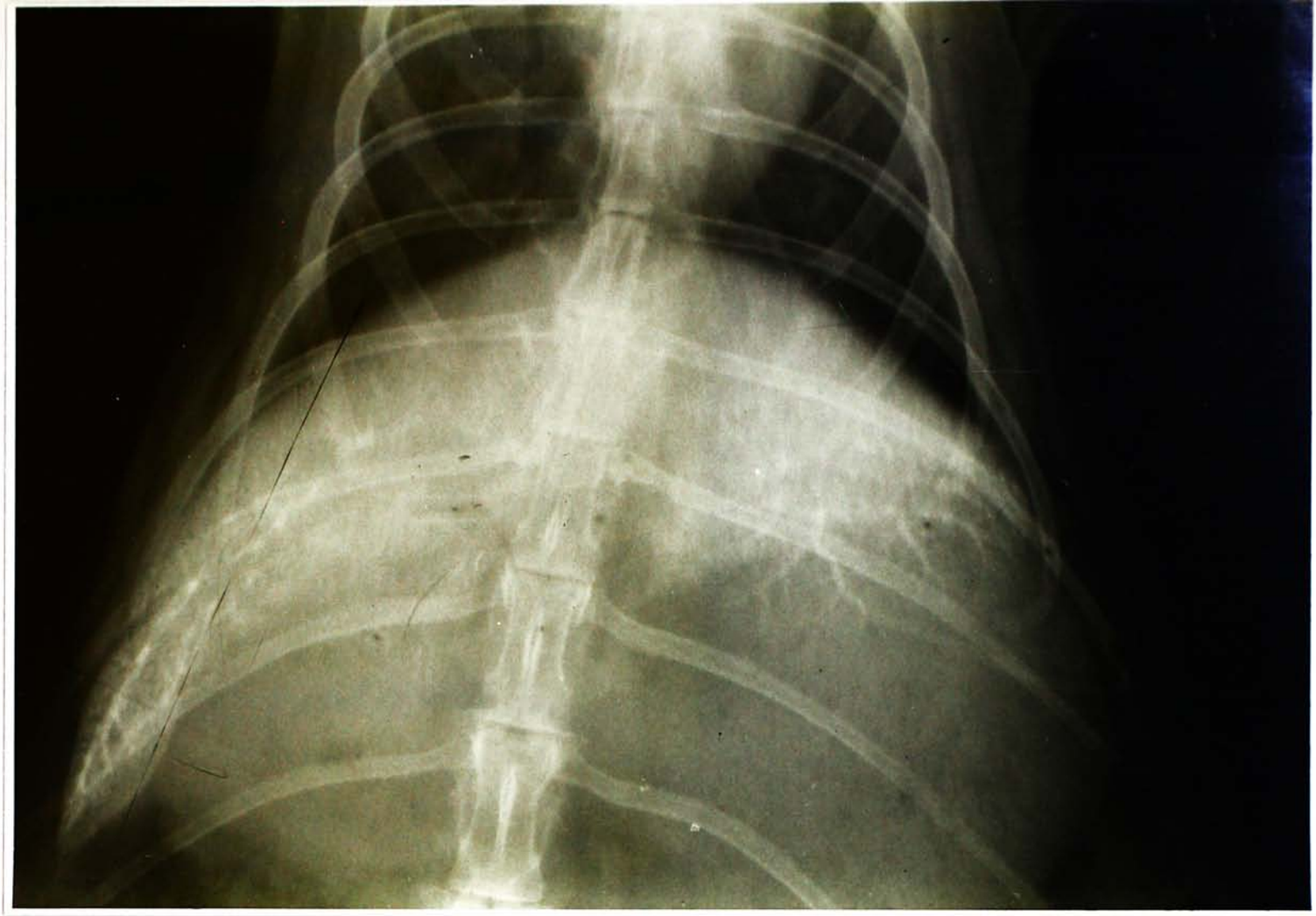


Fig. (4) - 7 : 1 minute after injection of Emulsion

Fig. (4) - 8 : 2 minutes after injection of Emulsion

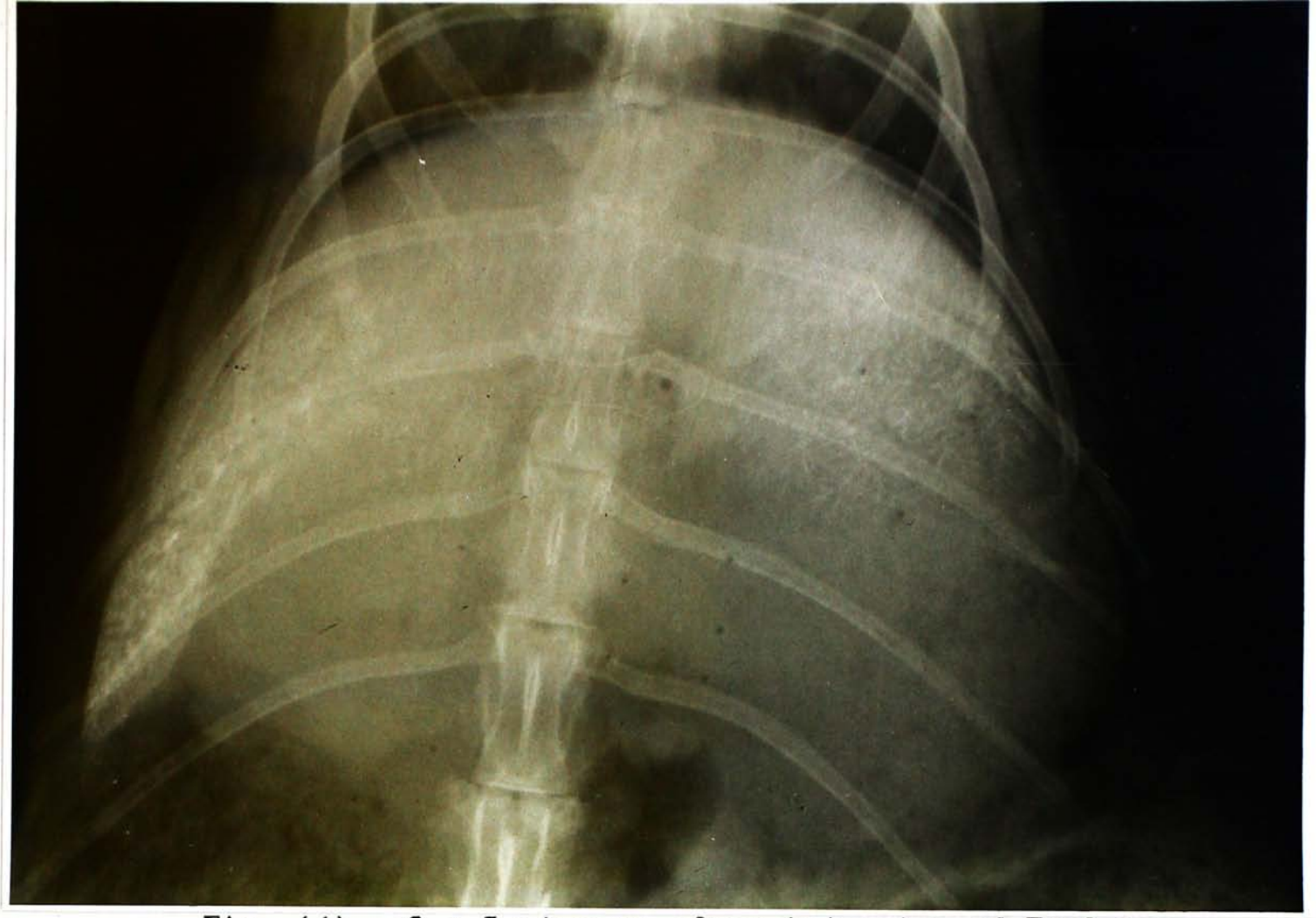


Fig. (4) - 9 : 5 minutes after injection of Emulsion

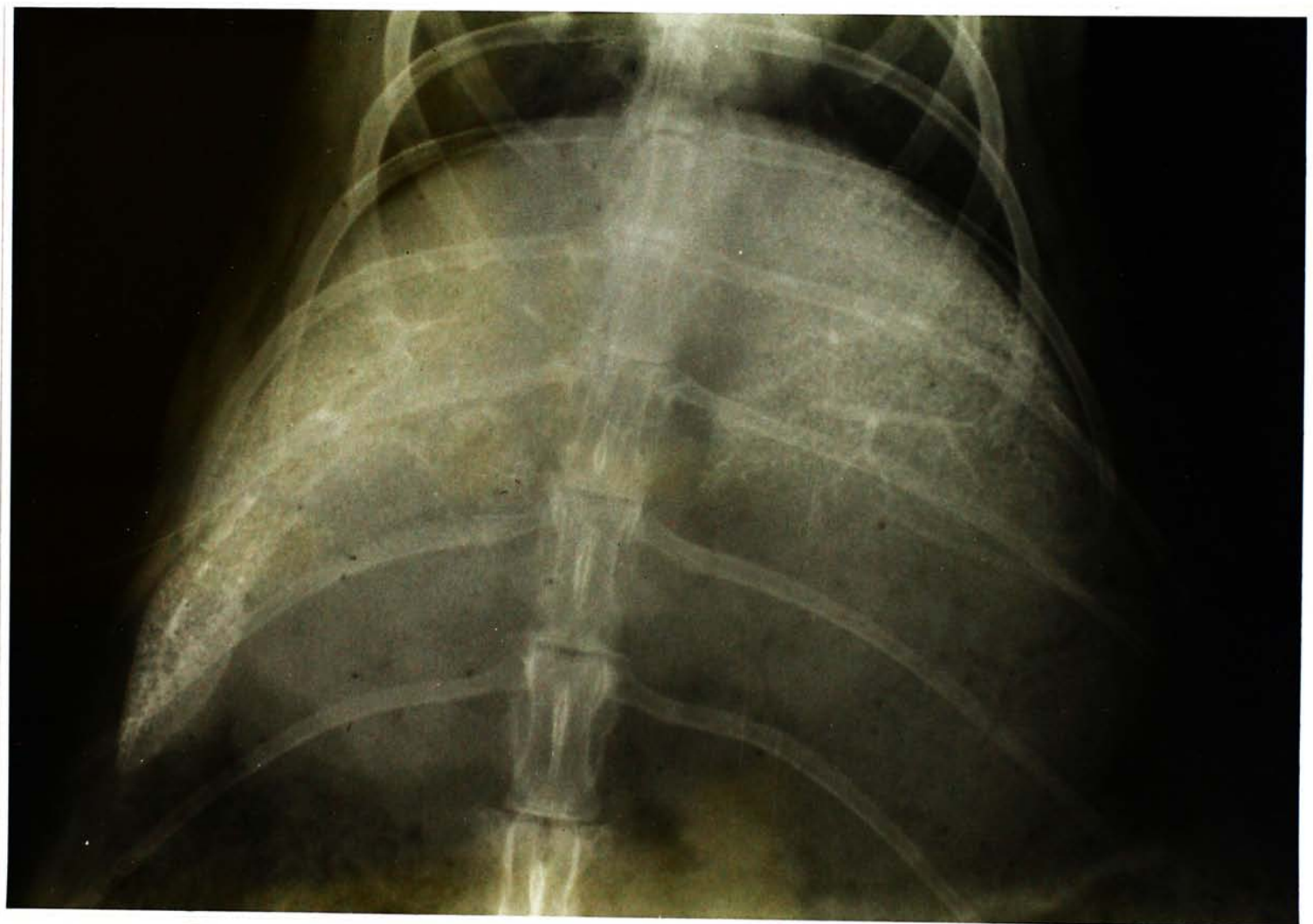


Fig. (4) - 10 : 10 minutes after injection of Emulsion

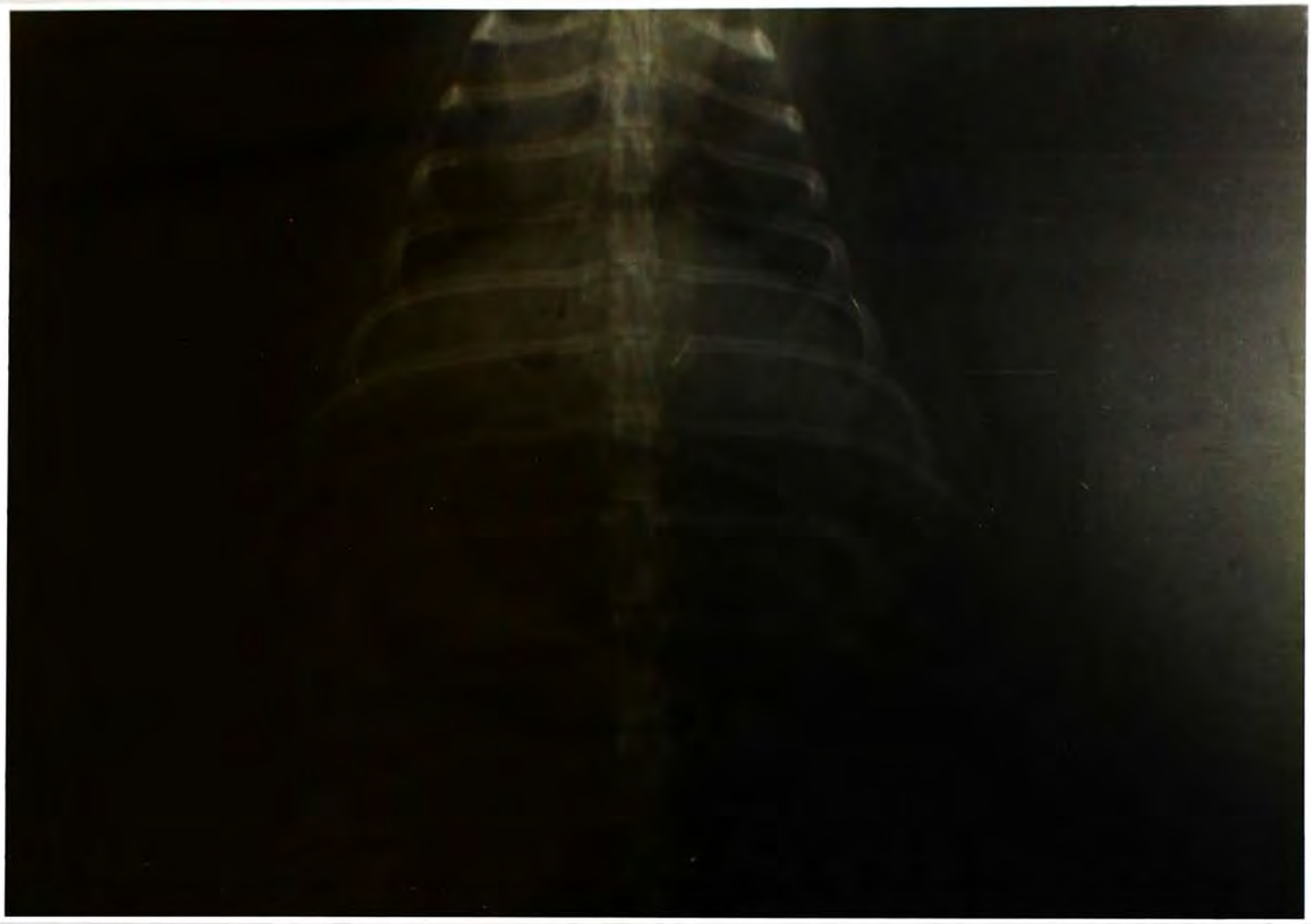


Fig. (4) - 11 : Control before injection of Epi-Lip mixture

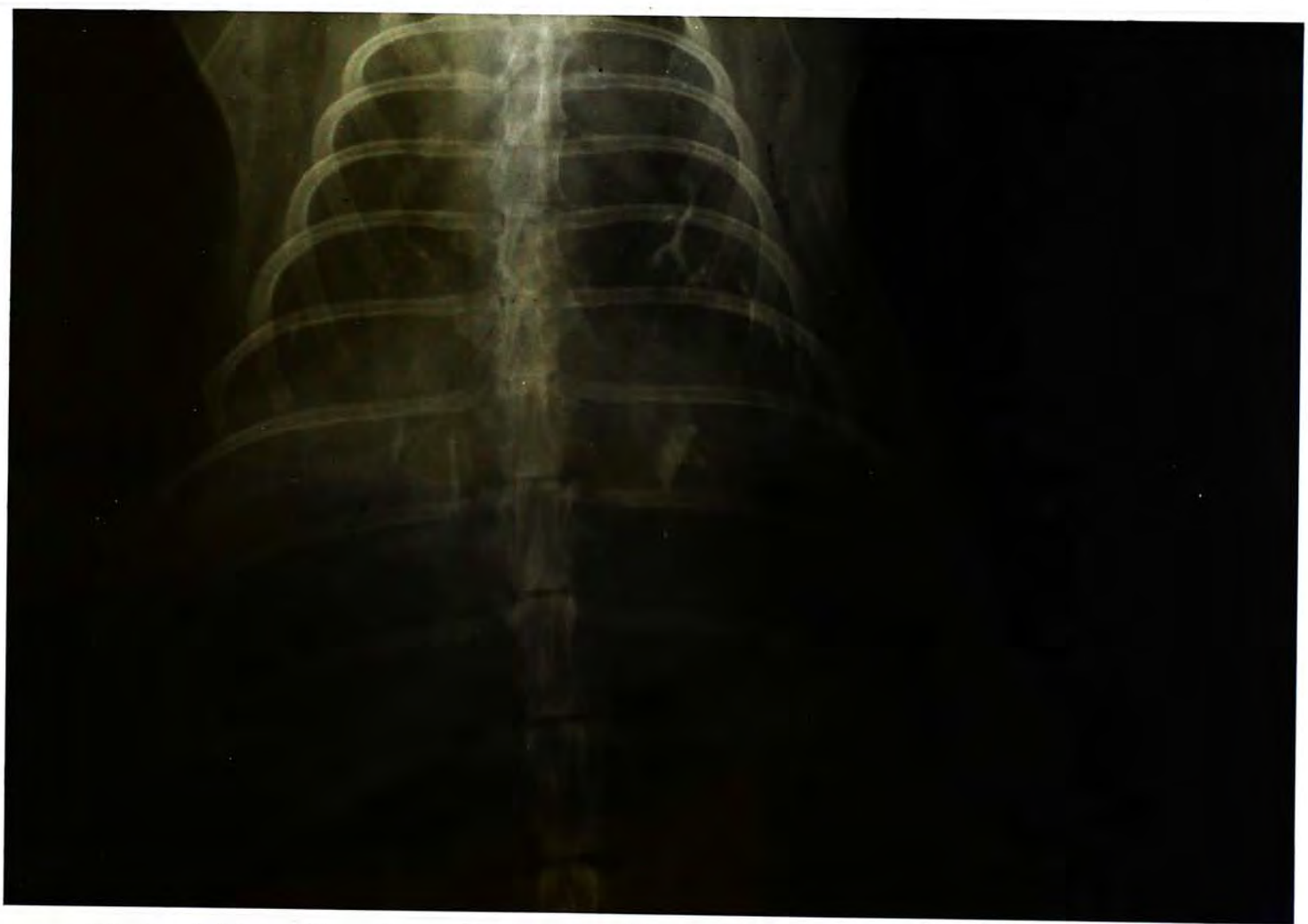


Fig. (4) - 12 Immediately after injection of Epi-Lip. mixture

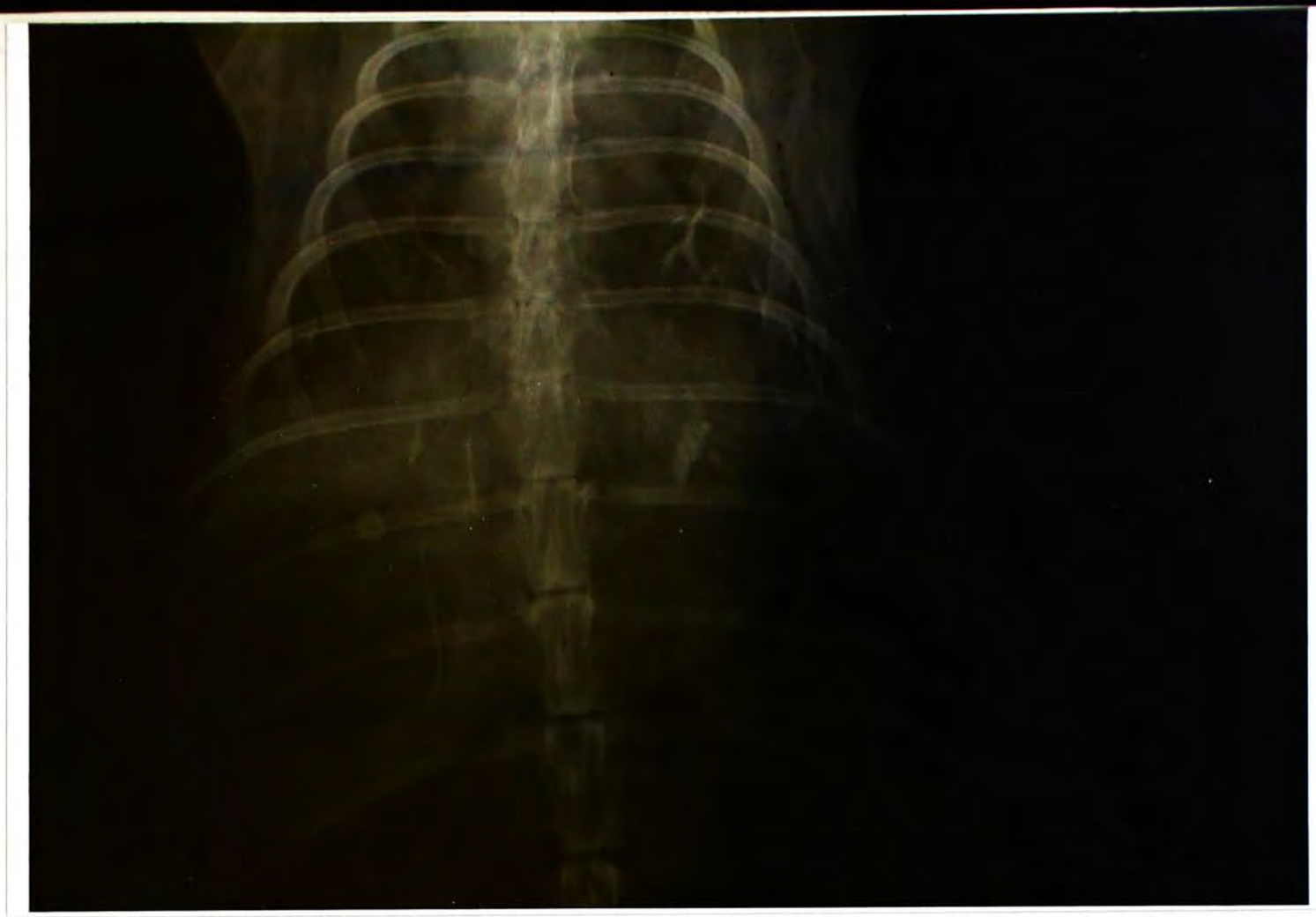


Fig. (4) - 13 : 1 minute after injection of Epi-Lip mixture

Fig. (4) - 14 : 2 minutes after injection of Epi-Lip mixture

Fig. (4) - 15 : 5 minutes after injection of epi-Lip mixture

Fig. (4) - 16 : 10 minutes after injection of Epi-Lip mixture

CHAPTER V : TOXICITY OF THE FORMULATED EMULSION

INTRODUCTION

Discussions in this Chapter are based on the formula of the emulsion designed in Chapter IV. It is as follows :

Lipiodol		25 - 50%
Polysorbate 20	}	
	}	5%
Sorbitan monolaurate	}	
Poloxamer		2%
Epirubicin		10 mg
Water for injection to		5 ml

Theoretically, every ingredient in the formulated emulsion should be tested for its toxicity. However, the individual acute toxicities of the active ingredient, epirubicin, and the system of emulsifiers have already been reported in literature (15, 47).

For epirubicin, the dose in man is 1 mg/Kg body weight as recommended by the original manufacturer (15). In animal studies, the range of LD50 in healthy mice when given intravenously was found to be 16 - 19 mg/Kg (48), and in dog, single doses of 2 mg/Kg were found to be lethal (49).

For the three emulsifiers, namely polysorbate -20, sorbitan monolaurate and poloxamer, toxicity data was established by the World Health Organisation (WHO)(47). The three emulsifiers are all frequently used in food stuffs as food additives, the estimated accepted daily oral intake for man is 0 - 25 mg/Kg body weight calculated as total sorbitan esters. In rat, the LD50 when given intravenously, was found to be 1,450 mg/Kg body weight and that for mice is 3,750 mg/Kg (47). This can be compared to the quantity incorporated in the formulated emulsion, that is, a total of 5% of polysorbate 20 and sorbitan monolaurate. For an animal of say, 4 Kg, according to the dosage regimen of 1 mg epirubicin/Kg, the total amount of polysorbate 20 and sorbitan monolaurate injected is only 100 mg and therefore should be well within the safety margin. For poloxamer, 2% is incorporated in the emulsion, only 80 mg is injected for an animal weighing 4 Kg and should therefore also be safe.

For Lipiodol, the recommended intramuscular dose when used as a lymphographic agent is 0.15 ml/Kg (42). In the formulated emulsion, 25% of Lipiodol is incorporated. According to the same dosage regimen of

epirubicin, 0.5 ml of the oil is given parenterally for a 4 Kg animal subject (compare with 0.6 ml as recommended) and therefore should also be within the acceptable safety margin. What needs to be established is whether there is an enhanced absorption into cells after incorporation in an emulsion, and if there is, whether it would cause any additional toxicity.

The above figures are calculated for ingredients in an emulsion according to each one's individual recommended dosages. As long as we are using an emulsion in our study, we need to assess the overall toxicity of the emulsion as a whole, to see whether there is any synergistic or antagonistic effect when the ingredients are mixed in such an emulsion.

The study of the acute toxicity of the overall emulsion is conducted in 2 parts : one with the vehicle alone and the other after the active ingredient epirubicin is added.

PART I : <u>ACUTE TOXICITY STUDIES OF THE VEHICLE</u>

A. Background

From the above discussion, it can be seen that the concentration of the individual emulsifying agents is within safety margin, therefore attention is only focused on the toxic effect, if any, of

Lipiodol when it is administered in the form of an emulsion.

Also as stated in the previous discussion, poloxamer 2% is added to the formula both as a stabilizing agent and for enhancement of tissue uptake of the drug (39). To find out the possible potentiated toxic effect when poloxamer is added, separate toxicity studies have to be performed.

Upon completion of this study, a formula with the lowest degree of toxicity can be determined.

B. Method

Different variations of the formulated emulsion were injected intravenously into the tail vein of mice and rats at increasing dosages. For rabbits, the emulsions were injected through the lateral ear vein. The number of deaths was recorded after 24 hours and the time of occurence was noted if possible. The surviving subjects were also weighed after 24 hours.

C. Results

Results of this study were assessed based on the normal dose of Lipiodol given as intramusculur injection : 0.15 ml/kg

(i) Formula (1)

Lipiodol		50%
Polysorbate 20	2.1% }	5%
Sorbitan monolaurate	2.9% }	
Water for injection to		100%

MICE

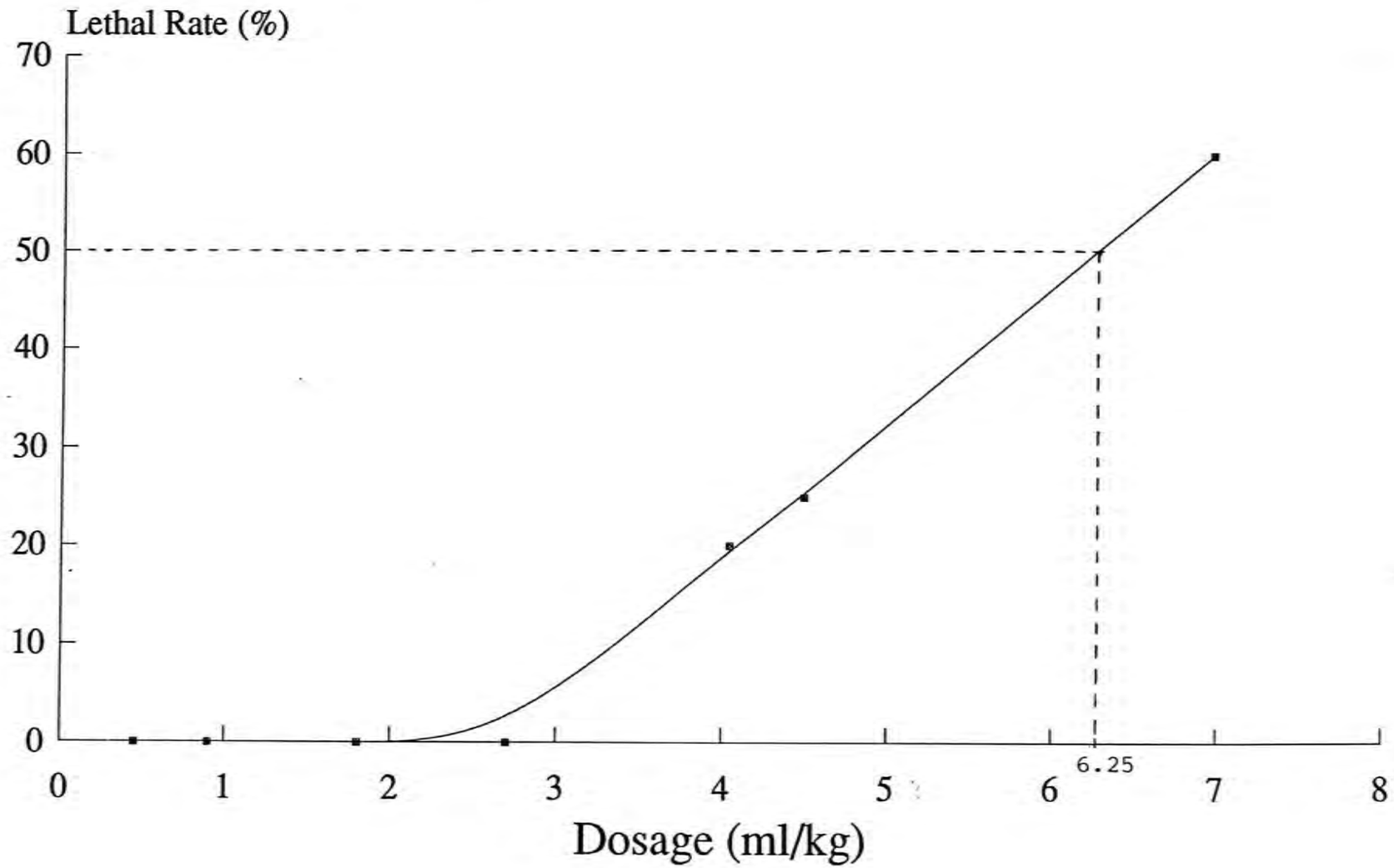
Dosage Used (ml/kg)	Times of normal dose of Lipiodol	No. tested	No. died	Lethal rate (%)
0.45	3	20	0	0
0.9	6	20	0	0
1.8	12	20	0	0
2.7	18	20	0	0
4.05	27	20	4	20
4.5	30	20	5	25
7.0	46.7	20	12	60

RATS

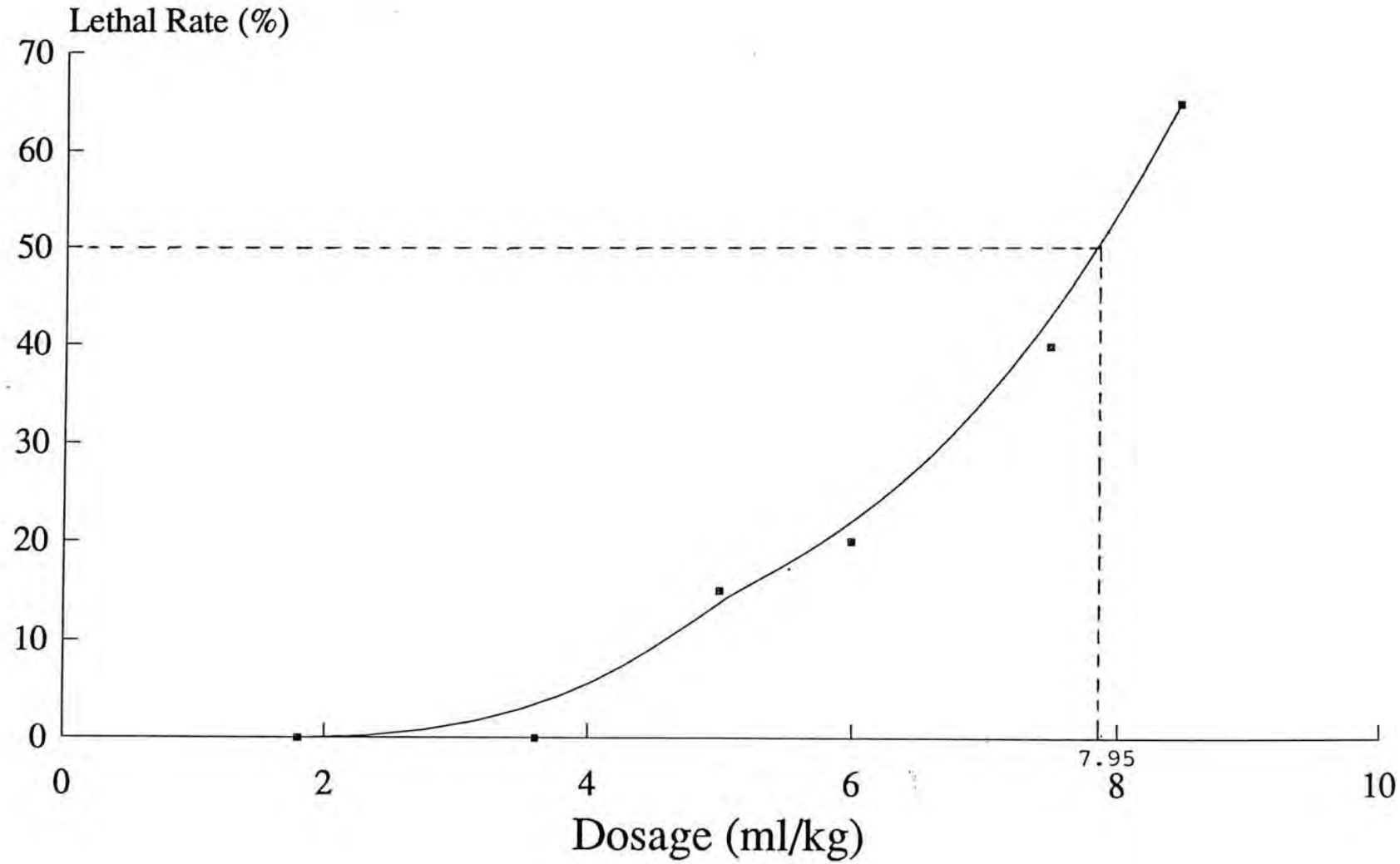
Dosage Used (ml/kg)	Times of normal dose of Lipiodol	No. tested	No. died	Lethal rate (%)
1.8	12	20	0	0
3.6	24	20	0	0
5.0	33.3	20	3	15
6.0	40	20	4	20
7.5	50	20	8	40
8.5	56.7	20	11	55

RABBITS

Dosage Used (ml/kg)	Times of normal dose of Lipiodol	No. tested	No. died	Lethal rate (%)
0.225	15	10	0	0
0.3	2.0	10	0	0
0.375	2.5	10	3	30
0.45	3.0	10	6	60
0.525	3.5	10	10	100



Figure(5)-1 : Formula (1)
TOXICITY IN MICE



Figure(5)-2 : Formula (1)
TOXICITY IN RATS

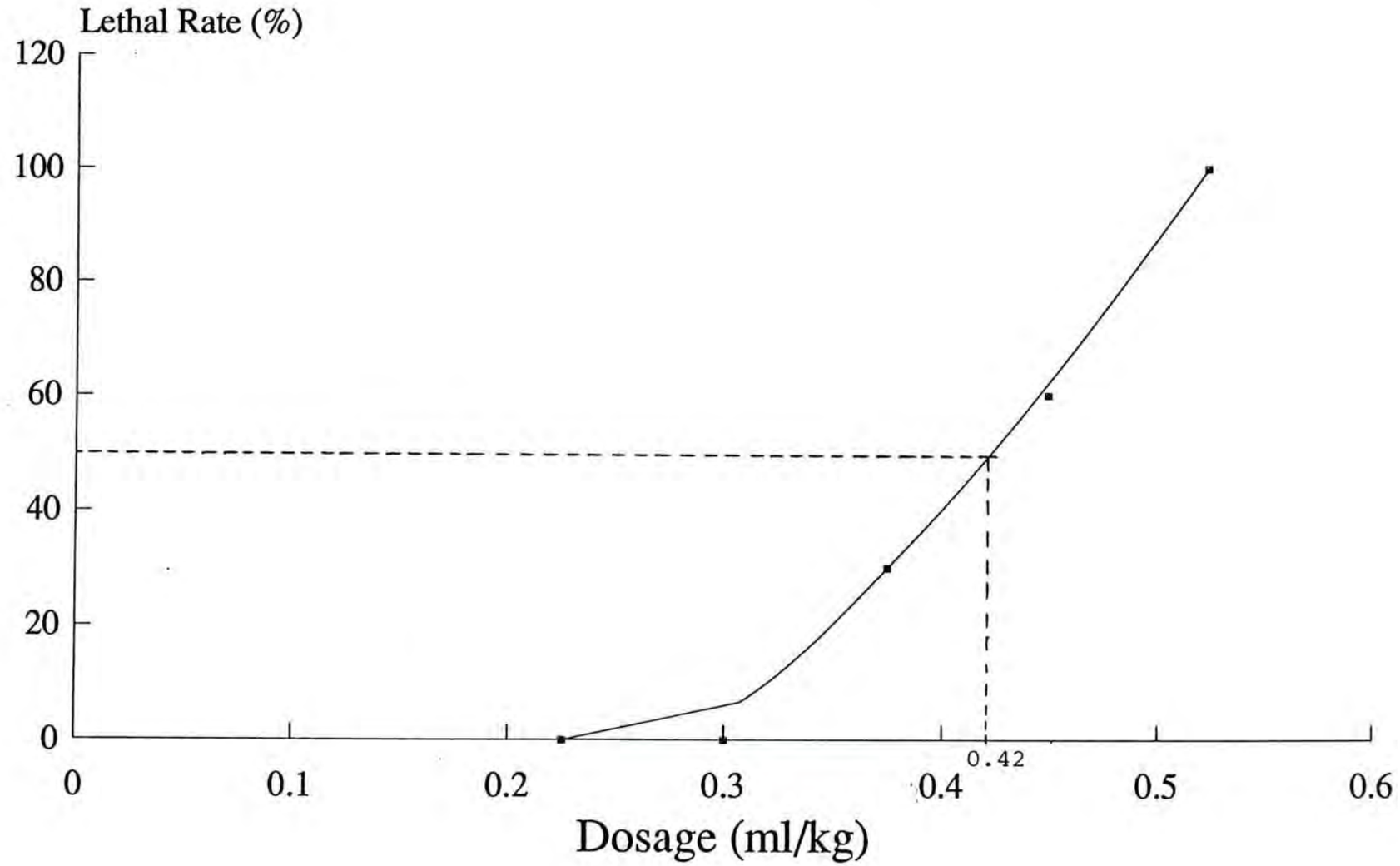


Figure (5)-3 : Formula (1)
TOXICITY IN RABBITS

Discussion:

From the graphs of Fig (5) - 1 to Fig (5) -3, it was found that when Lipiodol was given intravenously as an emulsion produced according to Formula (1),

LD - 50 (mice) = 6.25 ml/Kg
(43 times the normal dose)

LD - 50 (rats) = 7.95 ml/Kg
(53 times the normal dose)

LD - 50 (rabbits) = 0.42 ml/kg
(2.8 times the normal dose)

Data from this experiment suggested that the LD-50 of Lipiodol for mice and rats when given intravenously are similar and weights taken 24 hours after injection were also found to be similar as before. The LD 50 value found in rabbit is much lower, this is consistent with the observation in this study that rabbit has been more vulnerable to adverse effects of the drug than rat and mouse. The results obtained hence indicates toxicity of such a formulated emulsion is low.

(ii) Lipiodol 50% + Poloxamer 2% (mice, rats and rabbits)

Formula (2)			
Lipiodol	50%		
Polysorbate 20	2.1%	}	
		}	5%
Sorbitan monolaurate	2.9%	}	
Poloxamer	2%		
Water for injection to	100%		

Results :

MICE

Dosage Used (ml/kg)	Times of normal dose of Lipiodol	No. tested	No. died	Lethal rate (%)
0.15	1.0	20	0	0
0.3	2.0	20	0	0
0.6	4.0	20	0	0
0.75	5.0	20	5	25
0.9	6.0	20	17	85
1.05	7.0	20	20	100

RATS

Dosage Used (ml/kg)	Times of normal dose of Lipiodol	No. tested	No. died	Lethal rate (%)
0.15	1.0	20	0	0
0.3	2.0	20	0	0
0.6	4.0	20	2	10
0.75	5.0	20	8	40
0.9	6.0	20	19	95

RABBITS

Dosage Used (ml/kg)	Times of normal dose of Lipiodol	No. tested	No. died	Lethal rate (%)
0.15	1.0	10	0	0
0.225	1.5	10	5	50
0.3	2.0	10	10	100

Discussion :

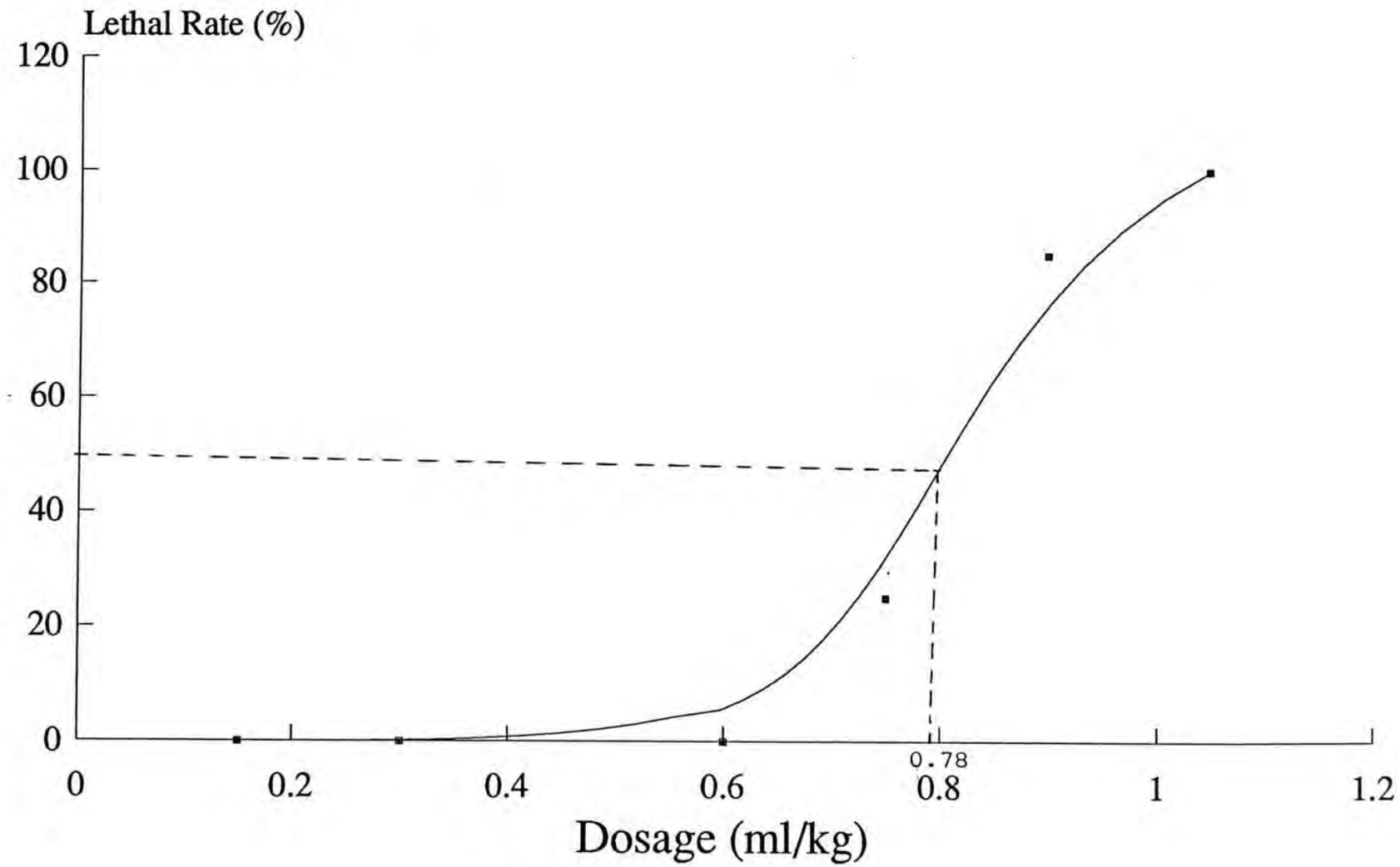
From the toxicity graphs (Fig (5)-4 to Fig (5)-6), it was found that

LD 50 (mice) = 0.78 ml/kg (5.2 times the normal dose)

LD 50 (rats) = 0.77 ml/kg (5.1 times the normal dose)

LD 50 (rabbits) = 0.225 ml/kg (1.5 times the normal dose).

It follows from the above findings that there is an obvious increase in toxicity after poloxamer is added to the formula. This is most likely due to an increased entry of oil into cells leading to necrosis or hemolysis. This can perhaps be eliminated by a reduction in the percentage of oil in the formula.



Figure(5)-4 : Formula (2)
TOXICITY IN MICE

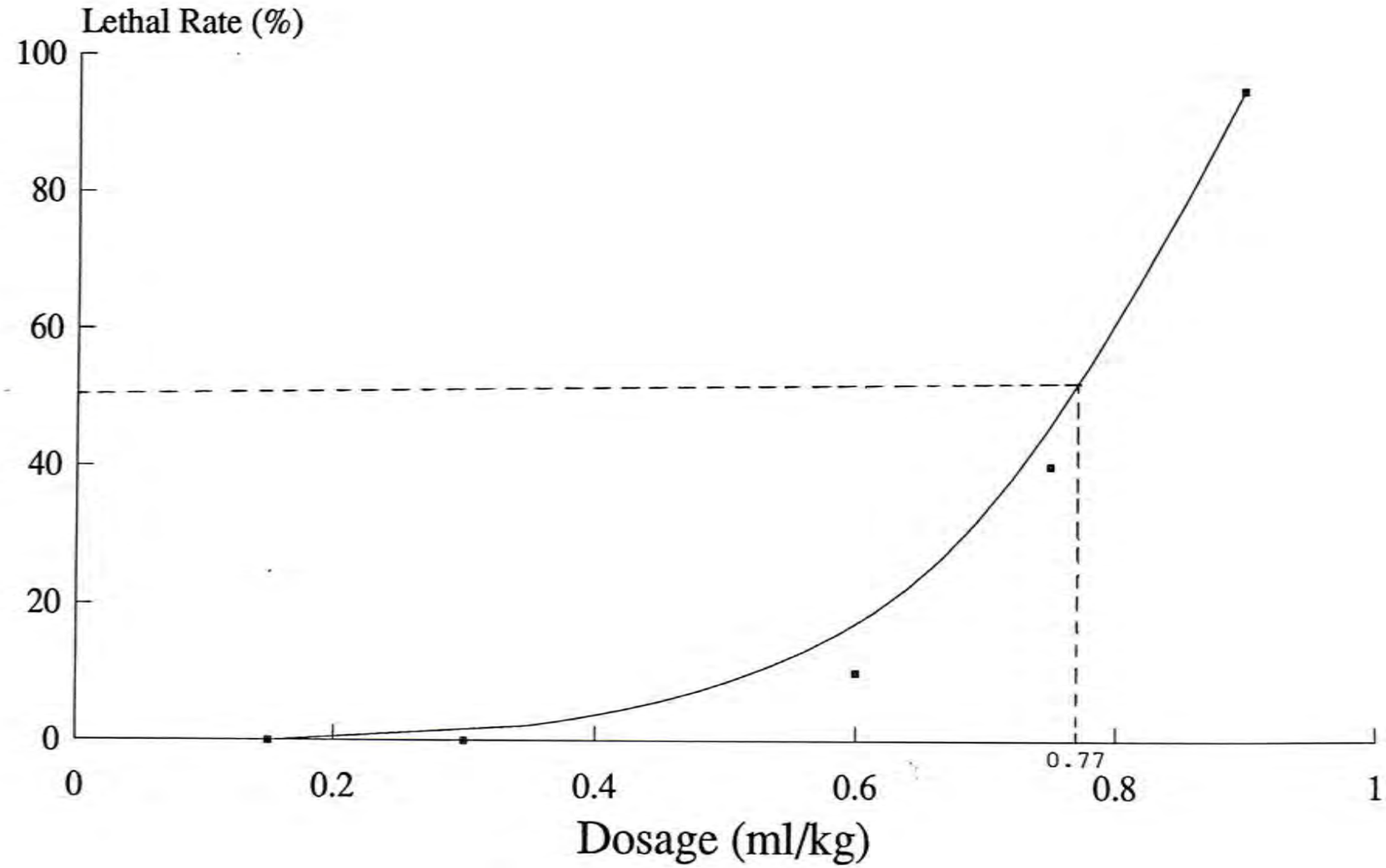
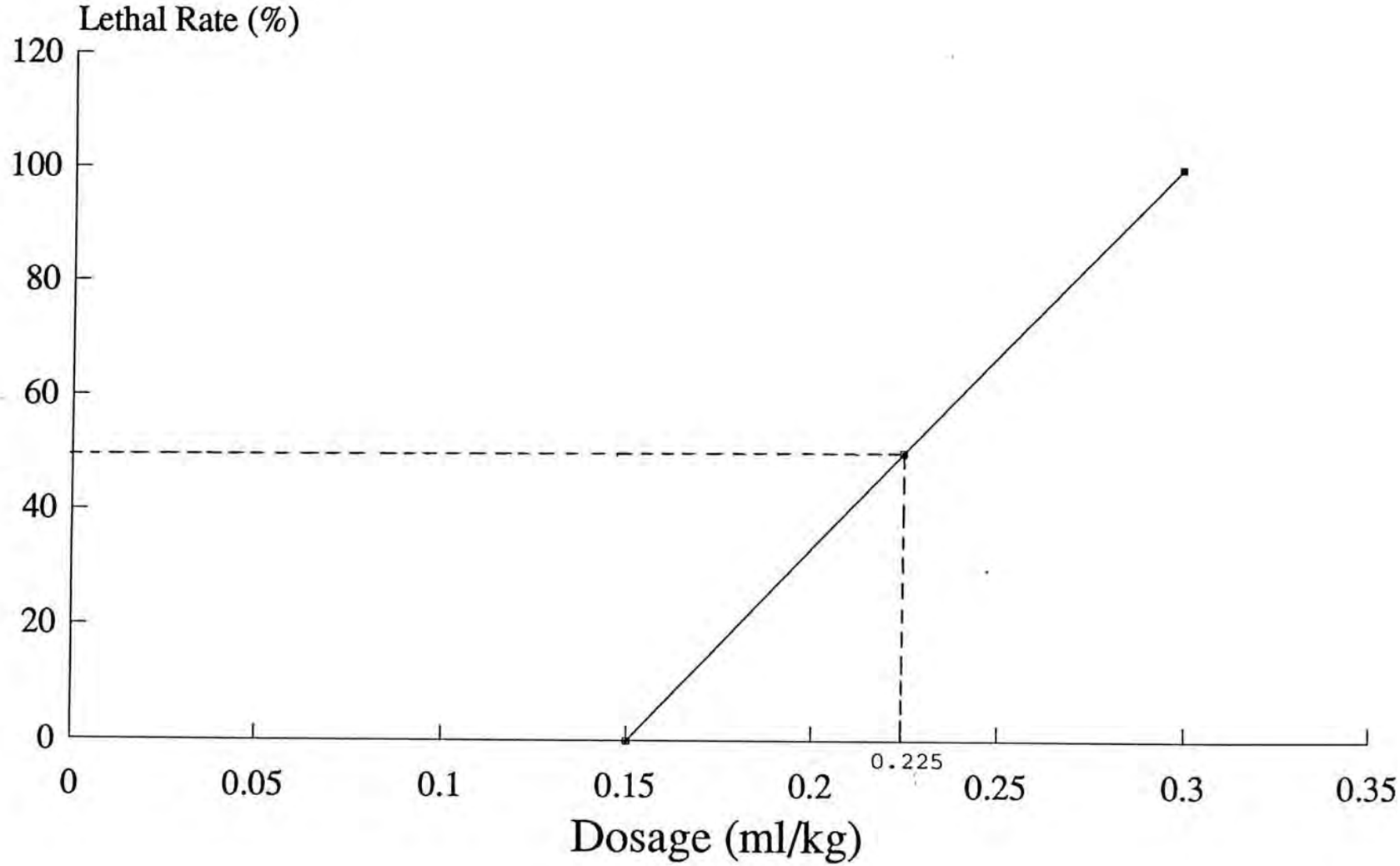


Figure (5)-5 : Formula (2) TOXICITY IN RATS



Figure(5)-6 : Formula (2)
TOXICITY IN RABBITS

(iii) Formula (3)

Lipiodol	25%		
Polysorbate 20	2.1%	}	
		}	5%
Sorbitan monolaurate	2.9%	}	
Poloxamer	2%		
Water for injection to	100%		

Results :

MICE

Dosage used (ml/Kg)	Times of normal dose	No. tested	No. died	Lethal rate (%)
0.15	1.0	20	0	0
0.3	2.0	20	0	0
0.45	3.0	20	0	0
0.6	4.0	20	7	35
0.75	5.0	20	10	50
0.9	6.0	20	18	90
1.05	7.0	20	20	100
1.2	8.0	20	20	100

RATS

Dosage used (ml/Kg)	Times of normal dose of Lipiodol	No. tested	No. died	Lethal rate (%)
0.15	1.0	20	0	0
0.3	2.0	20	0	0
0.45	3.0	20	0	0
0.6	4.0	20	6	30
0.75	5.0	20	7	35
0.9	6.0	20	9	45
1.05	7.0	20	17	85
1.2	8.0	20	20	100

RABBITS

Dosage used (ml/Kg)	Times of normal dose	No. tested	No. died	Lethal rate (%)
0.15	1.0	10	0	0
0.225	1.5	10	3	30
0.3	2.0	10	6	60
0.375	2.5	10	10	100

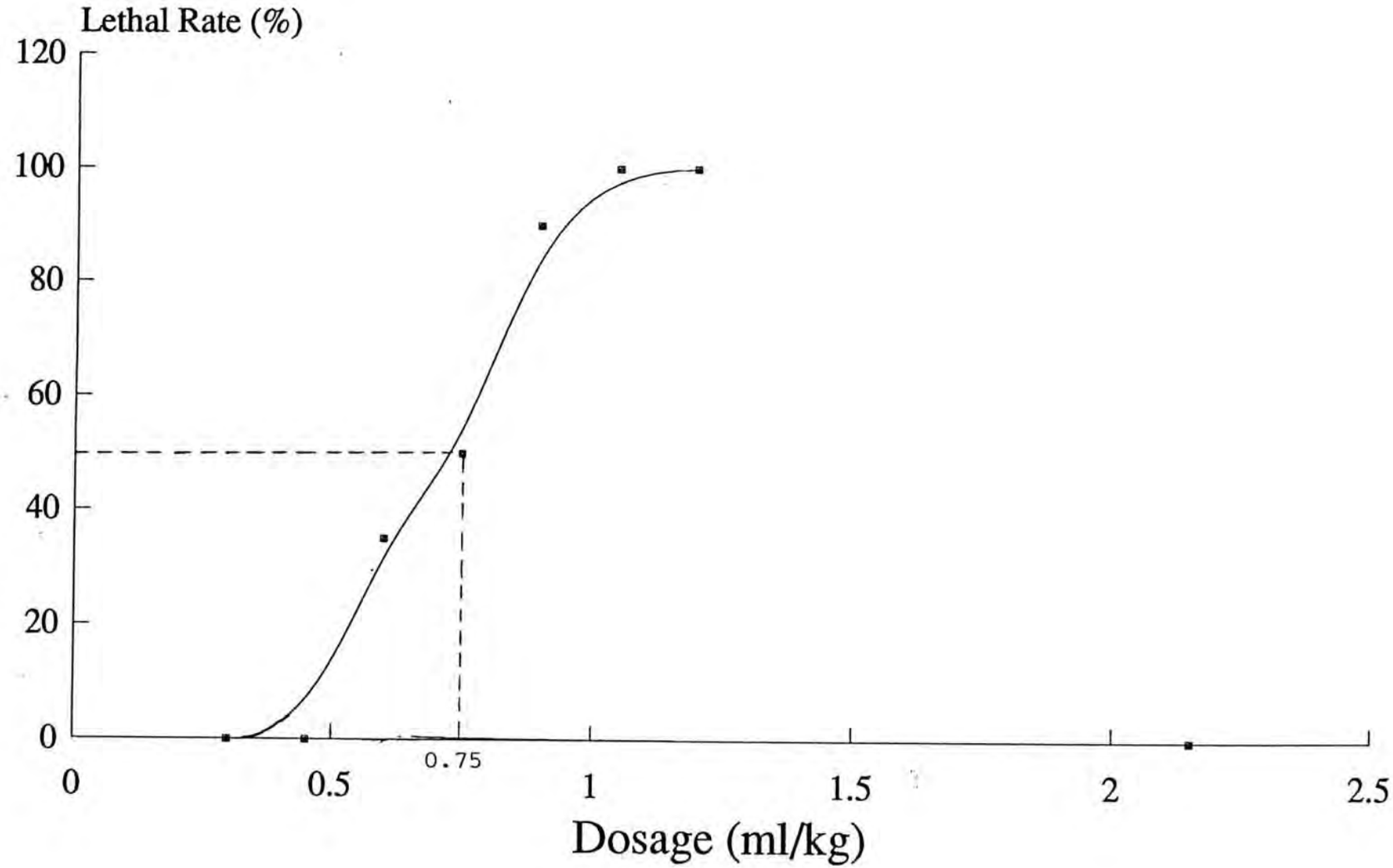


Figure (5)-7 : Formula (3)
TOXICITY IN MICE

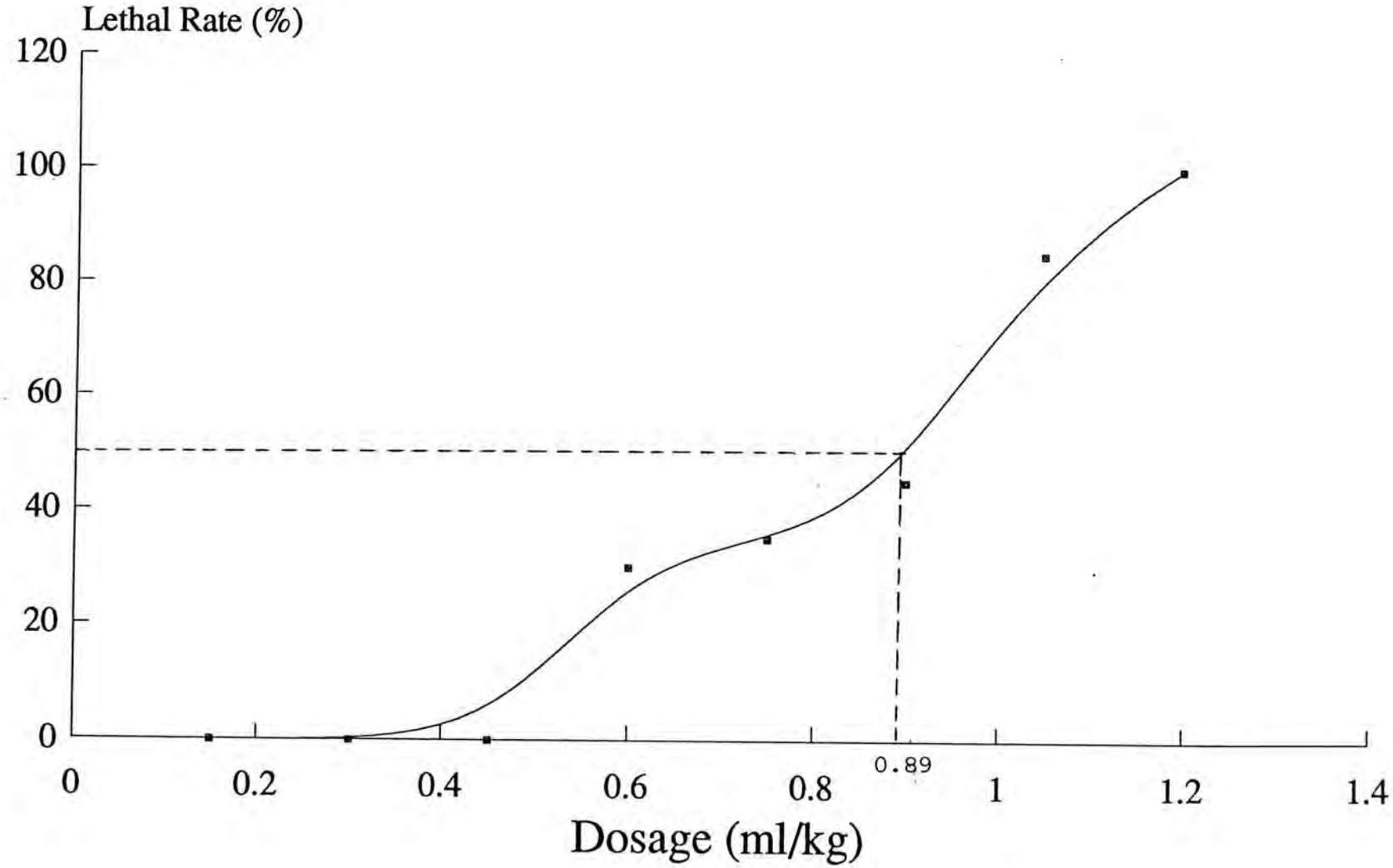
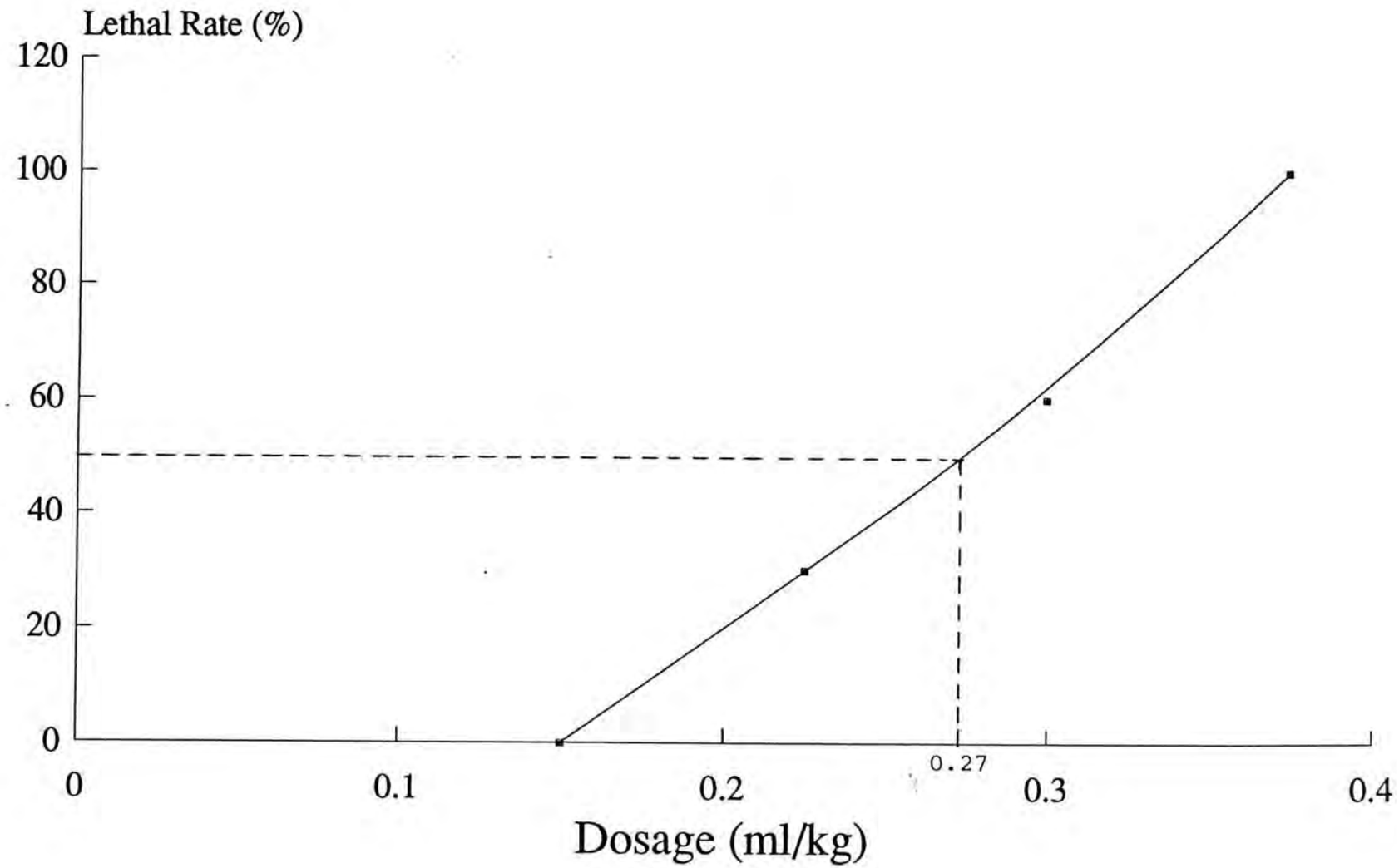


Figure (5)-8 : Formula (3) TOXICITY IN RATS



Figure(5)-9 : Formula (3)
TOXICITY IN RABBITS

From the graphs (Fig (5)-7 to Fig (5) -9), it was found that when Lipiodol was given intravenously as an emulsion produced according to Formula (3):

LD - 50 (mice) = 0.75 ml/kg (5 times the normal dose)

LD - 50 (rats) = 0.89 ml/kg (5.9 times the normal dose)

LD - 50 (rabbits) = 0.27 ml/kg (1.8 times the normal dose)

Discussion

Results from this study indicates that Formula (3) has a higher toxicity than Formula (1), but a slightly lower toxicity than Formula (2). Therefore there is evidence suggesting that the higher toxicity is due to the high percentage of oil. This could certainly be due to the presence of poloxamer as well. However, as poloxamer serves the dual purpose of being an emulsion stabilizer and facilitating the entry of lipid soluble drugs into cells, its benefits seem to be able to outweigh its slight risk.

Based on the above findings, the remaining parts of the research adopts Formula (3) as the standard formula for serving as a vehicle for antineoplastic agents.

PART II : ACUTE TOXICITY STUDIES OF THE EMULSION AFTER EPIRUBICIN IS ADDED

A. Background

The toxicity of epirubicin in animal is well-established as reported by the original manufacturer : intravenous LD50 in mice ranges from 16 - 19 mg/Kg (48), and single doses of 2 mg/Kg were lethal in dogs (49).

In this study, toxicity of epirubicin when given as an emulsion is studied using mice, rats and rabbits.

B. Method

An oil-in-water emulsion was produced based on the following formula :

Lipiodol	25%
Polysorbate 20	2.1%
Sorbitan monolaurate	2.9%
Poloxamer	2%
Epirubicin	10 mg
Water for injection to	5 ml

Increasing doses of the emulsion were injected into the tail vein of mice and rats and the lateral ear vein in rabbits. Results were recorded after 24 hours, and those surviving subjects were weighed.

C. Results

Results from this study were evaluated based on the normal dose of epirubicin : 1 mg/Kg as recommended by the manufacturer.

MICE

Dosage used (mg/Kg)	Times of normal dose of epirubicin	No. tested	No. died	Lethal rate (%)
1.0	1.0	20	0	0
2.0	2.0	20	0	0
2.5	2.5	20	0	0
3.0	3.0	20	0	0
3.5	3.5	20	0	0
4.0	4.0	30	9	45
4.5	4.5	30	15	50
5.0	5.0	20	15	75
5.5	5.5	20	18	90
6.0	6.0	20	20	100

RATS

Dosage used (mg/Kg)	Times of normal dose of epirubicin	No. tested	No. died	Lethal rate (%)
1.0	1	10	0	0
2.0	2	10	0	0
3.0	3	10	0	0
4.0	4	10	3	30
4.5	4.5	10	4	40
5.0	5	10	6	60
5.5	5.5	10	8	80
6.0	6	10	10	100

RABBITS

Dosage used (mg/Kg)	Times of normal dose	No. tested	No. died	Lethal rate (%)
1.0	1.0	10	0	0
1.5	1.5	10	0	0
2.0	2.0	10	1	10
2.5	2.5	10	6	60
3.0	3.0	10	10	100

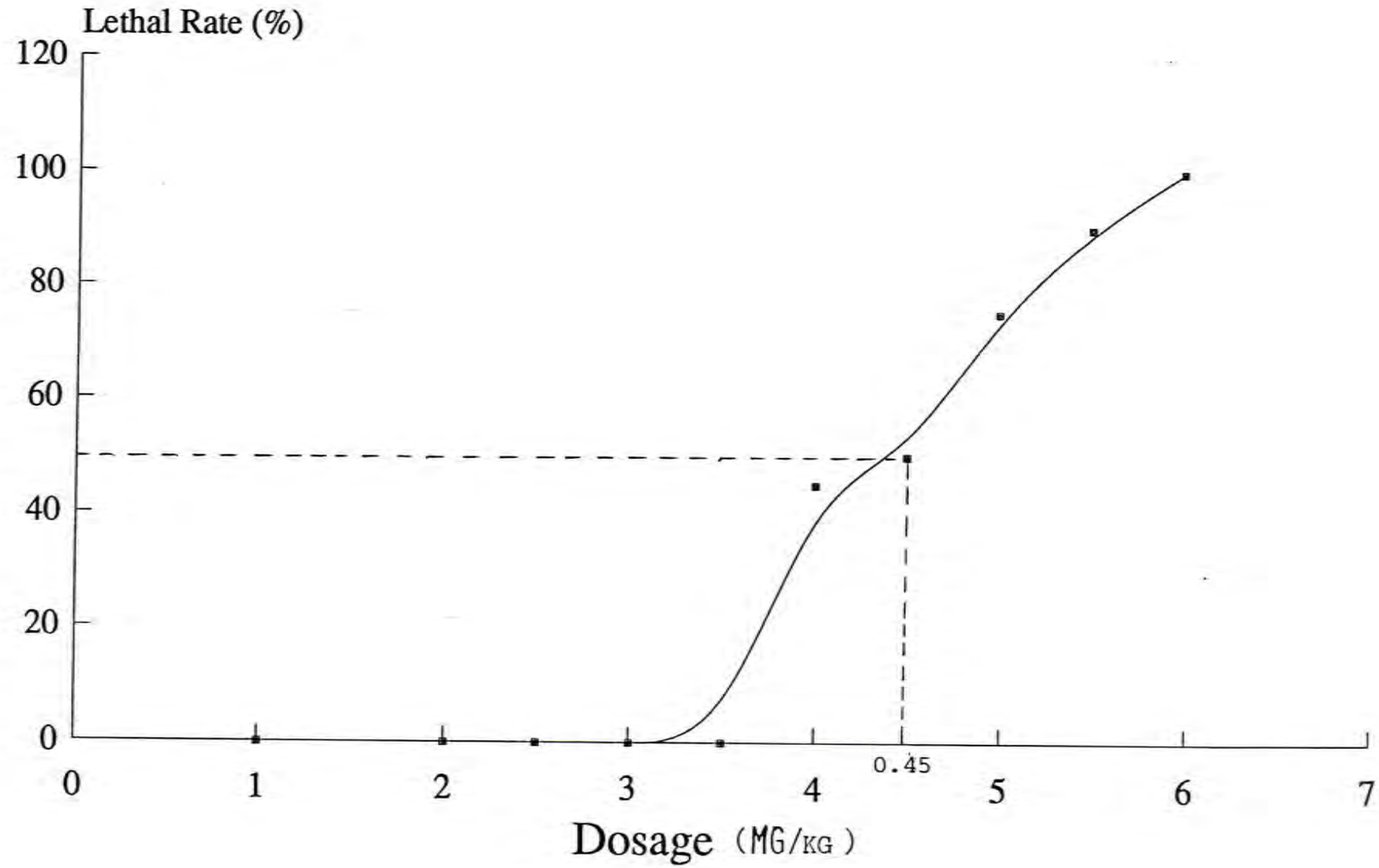


Figure (5)-10
TOXICITY IN MICE

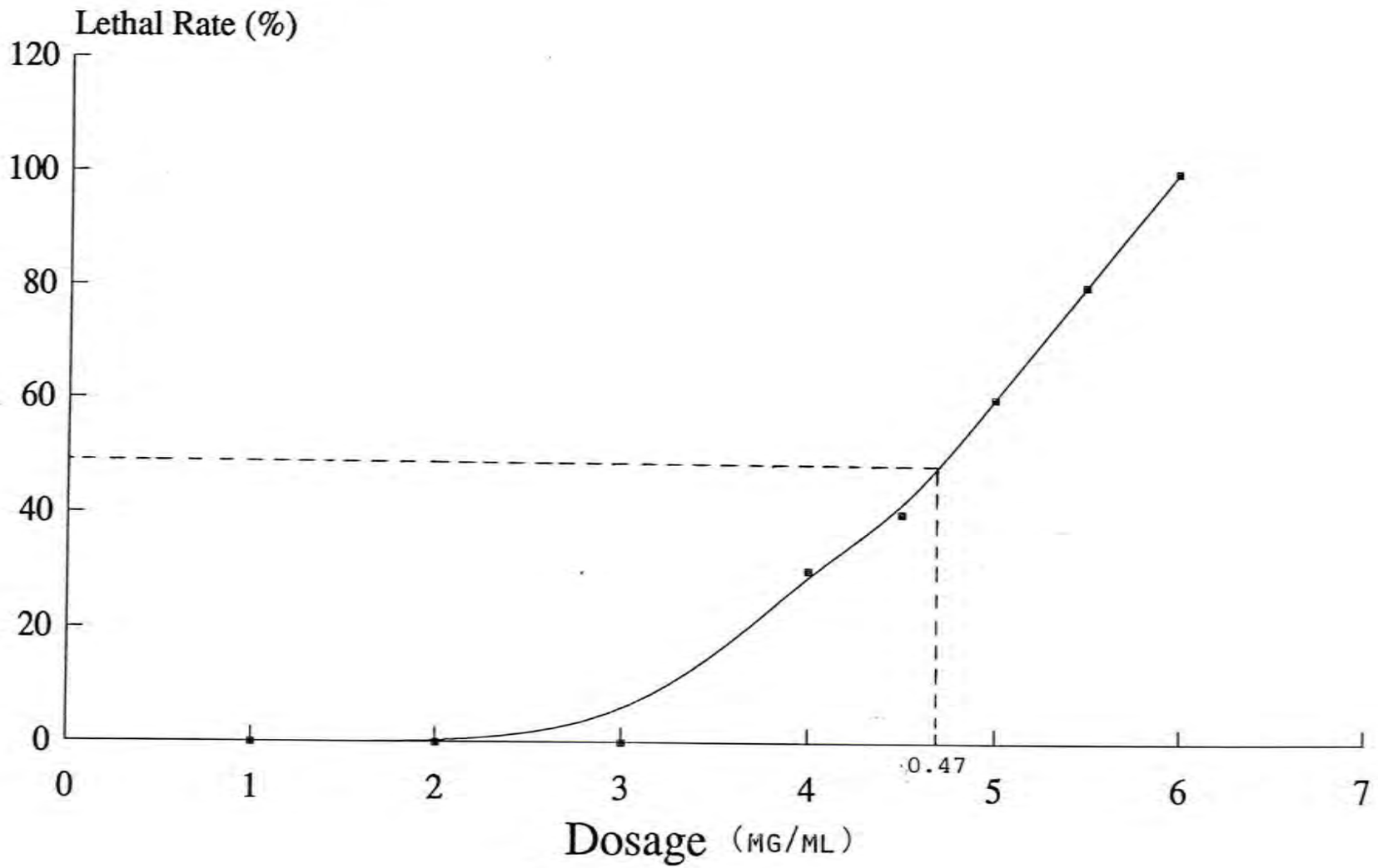


Figure (5)-11
TOXICITY IN RATS

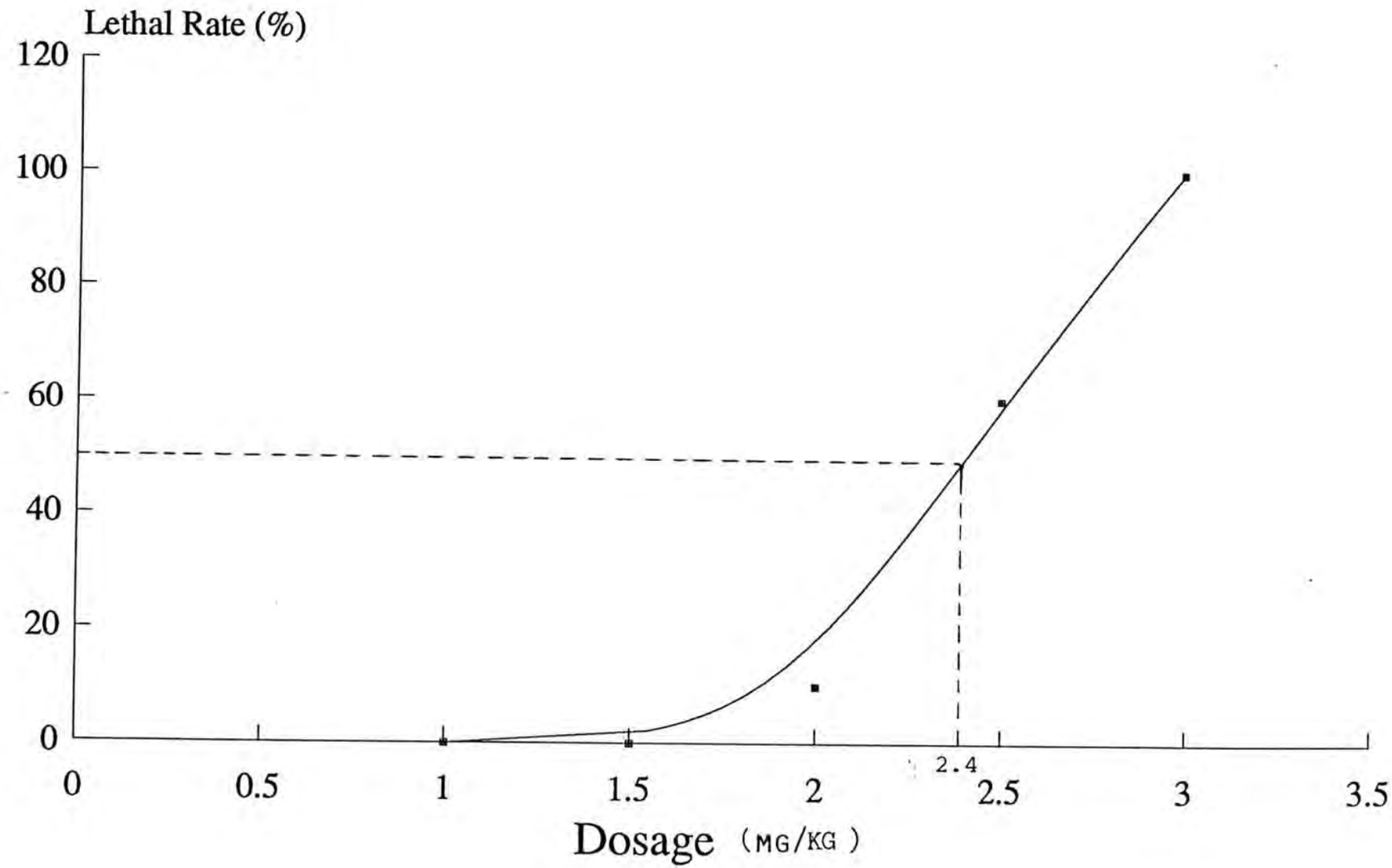


Figure (5)-12
TOXICITY IN RABBITS

Disussion:

From Fig (5)-10 to Fig (5)-12, it was found that for epirubicin emulsion :

LD 50 (mice) = 4.5 mg/kg (4.5 times the normal dose)
LD 50 (rats) = 4.7 mg/kg (4.7 times the normal dose)
LD 50 (rabbits) = 2.4 mg/kg (2.4 times the normal dose)

Although the result in mice indicates a higher toxicity of epirubicin when given as an emulsion as compared to an aqueous solution: LD50 of 4.5 mg/Kg vs 16 -19 mg/Kg (48), it should still be safe when normal doses are given.

From Fig. (5) - 12, it can be seen that the LD -50 for epirubicin emulsion when given intravenously in rabbits is 2.4 mg/kg. This is some what lower than in mice, but bearing in mind that rabbit was observed to be more vulnerable to adverse effects of drug in the study, a LD - 50 which is only twice the normal dose is still acceptable.

To conclude, the formulated emulsion appears to be safe for intravenous administration into the animal subjects, and is therefore adopted for subsequent kinetic studies in rabbits.

CHAPTER VI :

THE PHARMACOKINETIC STUDIES OF THE FORMULATED EPIRUBICIN EMULSION IN RABBITS

INTRODUCTION

From Chapter III, we notice the results of directly mixing epirubicin aqueous solution with Lipiodol were not pharmaceutically or clinically satisfactory. In this chapter, studies were done by injecting the formulated epirubicin emulsion firstly intravenously, and secondly intrahepatically into the hepatic-portal vein of rabbits. The results are compared with those using plain epirubicin solution.

PART I: INTRAVENOUS INJECTION OF EPIRUBICIN EMULSION VS EPIRUBICIN AQUEOUS SOLUTION

1. Method

Cannulation was performed on the lateral vein and the central artery of the ear of 10 rabbits. Pentobarbitone 40 mg/kg was injected intravenously to produce anesthesia. Epirubicin aqueous solution, in dosage of 1 mg/kg (49), was injected also intravenously

after 5 -10 minutes, Blood samples were collected from the central artery at 0, 2.5, 5, 10, 15, 30, 60, 120, 240, and 360 minutes after administration. Afterwards the cannulas were withdrawn and the surgical wounds were cleansed with alcohol 75%, and was securely wrapped with dressing. The subject was then brought back to the holding place followed by normal feeding. The same procedure was done with epirubicin emulsion using the same dosage. Extraction and quantitation by HPLC were performed according to methods described in Chapter II. Results were analysed using pharmacokinetic principles.

PART II : INTRAHEPATIC INJECTION OF EPIRUBICIN EMULSION VS EPIRUBICIN AQUEOUS SOLUTION

Method

Cannulation was done on the lateral vein and the central artery of the ear of 8 rabbits. Pentobarbitone 40 mg/kg was used to anesthesize the subjects by intravenous injection.

An incision was made on the abdomen of the rabbit. After a side-branch of the hepatic portal vein was located, epirubicin emulsion, in the dosage of 1 mg/kg, was injected slowly over one minute. Afterwards a bull-dog clamp was used to stop bleeding from the vein after the needle and syringe were withdrawn. Suture was done on the incision site and the subject was maintained in an anesthesized state. Blood samples were collected from the central artery at 0, 2.5, 5, 5, 10, 15, 30, 60, 120, 240 and 360 minutes after administration. As cross-over study could not be performed on an operated rabbit, it was destroyed at the end of the experiment.

Exactly the same procedure was carried out in 10 separate rabbits but using epirubicin aqueous solution. Extraction and quantitation by HPLC were performed on the samples as described in Chapter II. Results were analysed using pharmacokinetic principles.

RESULTS

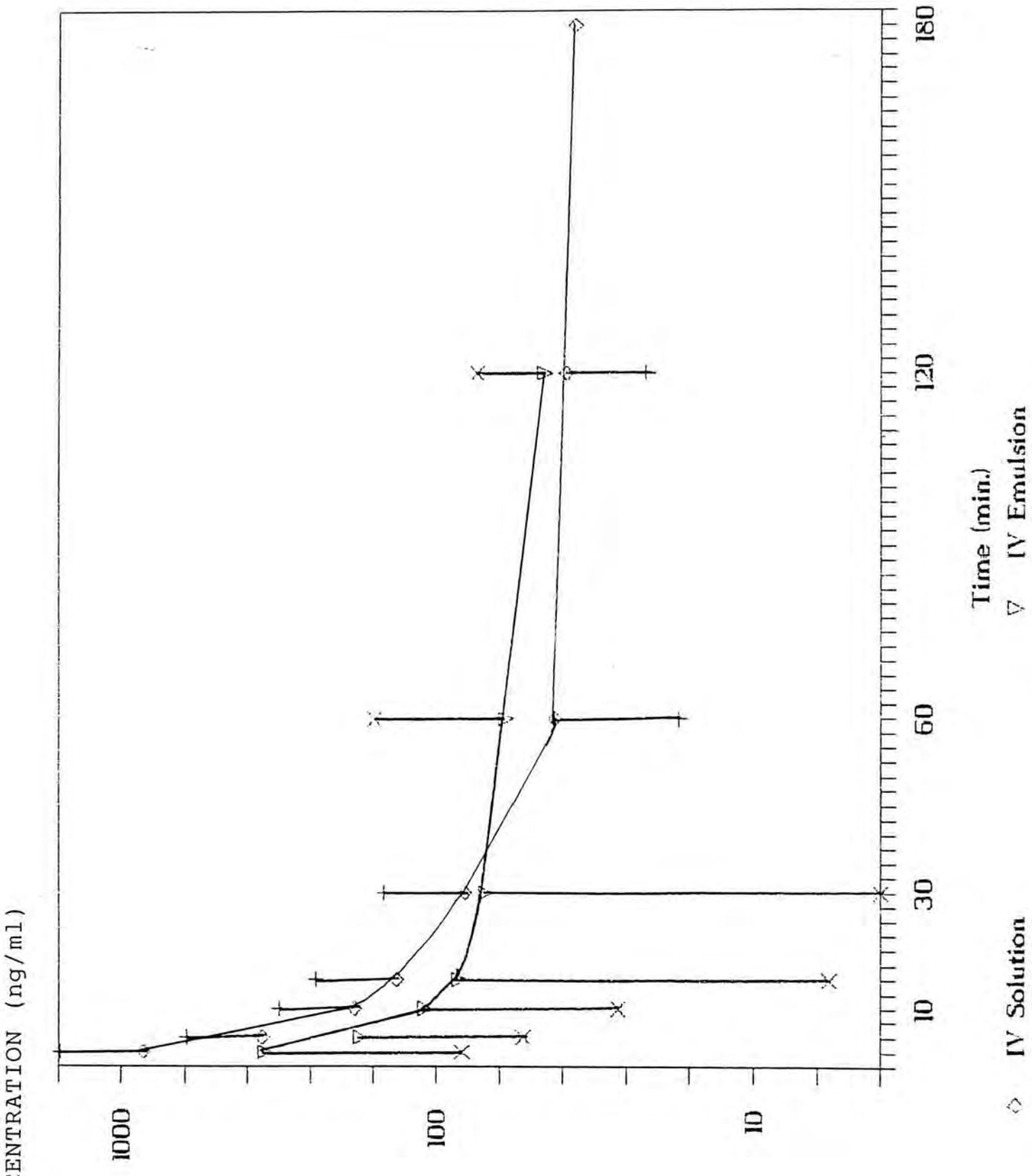
The plasma concentrations after the 2 different routes of administration of the 2 different dosage forms are shown in Appendix (III).

1. **Comparison of disposition between IV epirubicin solution and IV epirubicn emulsion**

Figure (6) - 1 compares the mean serum concentration - time profile of epirubicin in rabbits after IV administration of emulsion and solution, and Table (6) - 1 summarises the mean kinetic parameters. Some obvious differences were observed between the 2 groups of subjects. The AUC_0^{∞} is higher after IV solution while clearance is slower, the apparent volume of distribution smaller and elimination half-life and mean residence time longer.

	IV Emulsion (n = 10)	IV Solution (n = 10)	P<
AUC_0^{∞} (ng ml^{-1} min)	8054.7 (9003.9)	10846.1 (8731.9)	0.05
Cl (ml min^{-1} Kg^{-1})	1561.9 (1422.6)	812.8 (687.7)	0.05
Vss (ml Kg^{-1})	48783.2 (35956.9)	30908 (26532.8)	0.05
MRT (min)	50.1 (30.3)	75.8 (125.6)	0.05
$T^{1/2}$ (min)	37.7 (20.2)	63.4 (92.4)	0.05

Table (6) - 1 : Mean (±SD) kinetic parameters of epirubicin after intravenous (IV) injection of emulsion and plain solution



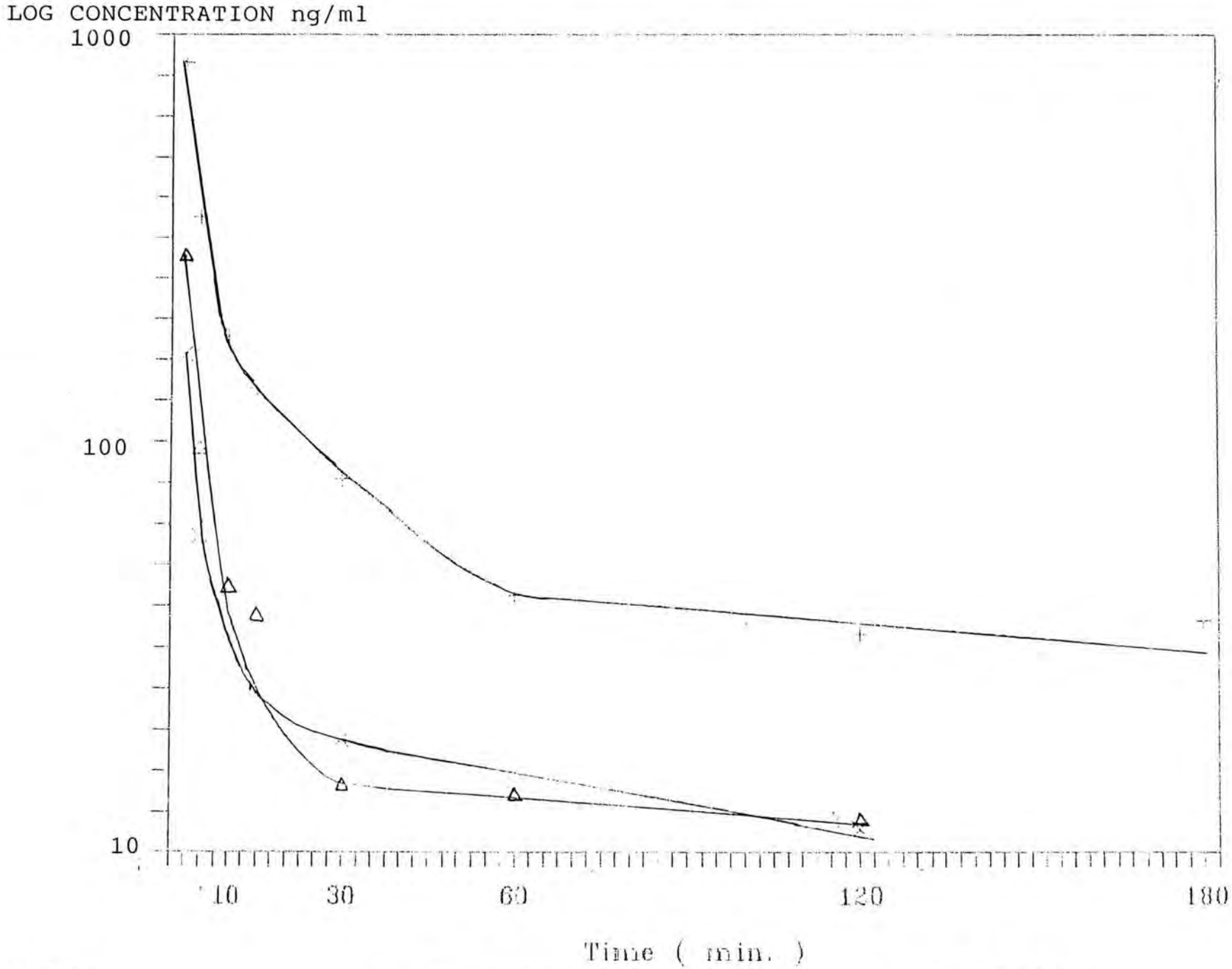
Fig(6)-1 : COMPARISON OF DISPOSITION OF EPIRUBICIN AFTER IV INJECTIONS OF EMULSION AND SOLUTION

2. **Comparision of disposition between IH injection of solution, emulsion and IV injection of solution of epirubicin.**

Figure (6) - 2 compares the mean serum concentration-time profile after the 3 different modes of administration and Table (6) - 2 summarizes the kinetic parameters. Intrahepatic injection of emulsion has the lowest AUC, but clearance is the fastest with the highest apparent volume of distribution. Though the values of half-lives do not differ to a significant degree, IH emulsion apparently has the shortest half-life. Bioavailabilty of IH injections is less than 50% indicating a high first-pass clearance by the liver.

	IH emulsion (n = 8)	IH solution (n = 8)	IV solution (n = 10)	P<
AUC_0^∞ ($ng\ ml^{-1}\ min$)	2185 (1626.1)	3931.2 (1926.4)	10846.1 (8731.9)	0.05
Cl ($ml\ min^{-1}\ Kg^{-1}$)	3778 (3140.4)	2701.8 (2406.4)	812.8 (687.7)	0.02
Vss ($ml\ Kg^{-1}$)	136673.8 (80210.5)	128653.7 (89438.4)	30908 (26532.8)	0.05
MRT (min)	66.6 (55.6)	83.1 (75.7)	75.8 (125.6)	0.05
T1/2 (min)	52.1 (45.1)	68.4 (59.3)	63.4 (92.4)	0.05
Bioavailability	0.2	0.36	1.0	

Table (6) - 2 : Mean(±SD) Kinetic parameters of epirubicin after IH emulsion, IH solution and IV solution.



Fig(6)-2 : COMPARISON OF DISPOSITION OF EPIRUBICIN AFTER IH EMULSION, IH SOLUTION, AND IV SOLUTION

Discussion

The study reveals some important differences of kinetic parameters in rabbits after IV and IH administration of plain epirubicin solution and formulated epirubicin emulsion.

When intrahepatic injection of emulsion is compared to IV injection of solution and emulsion, a significant degree of retention is demonstrated. Differences in bioavailability also suggest some degree of drug targeting is achieved. This might be even more obvious if hepatoma was present because neoplasms usually lack a clearing or drainage system resulting in accumulation of drugs in cytoplasm of cells. This is an obvious improvement in the design of the formulated emulsion over the epirubicin-Lipiodol mixture used previously.

It is also important to note that the elimination half-life of intrahepatic injection, whether emulsion or solution, is comparatively shorter. This could be due to the first pass metabolism of the drug after intrahepatic administration, as epirubicin is mainly metabolized by liver.

The lower AUC_{0}^{∞} after intrahepatic injection may suggest that the amount of epirubicin in the general circulation is lower, and this may be related to lower systemic side effect of the drug. Thus, targeting hepatoma with the formulated epirubicin emulsion may enable hepatic administration of large dose of epirubicin with perhaps less systemic side effects.

In conclusion, the formulated emulsion demonstrates some important superior characteristics when compared with the previous epirubicin - Lipiodol mixture. Further investigation and assessment are worthwhile for future application in human subjects.

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Appendix 1

Calibration of assay for Epirubicin and 13-epidoxorubicin (13-OH)

A. Epirubicin peak-height ratios :

Conc. (ng/ml)	1	2	3	4	5	6	Mean
125	0.05	0.11	0.06	0.06	0.06	0.05	0.07
250	0.15	0.19	0.2	0.15	0.18	0.15	0.17
500	0.28	0.25	0.32	0.31	0.29	0.35	0.3
1000	0.57	0.48	0.55	0.6	0.4	0.4	0.5
2000	1.09	1.1	1.03	1.01	1.13	1.06	1.07

B. 13 - Epirubicin peak-height ratios :

Conc. (ng/ml)	1	2	3	4	5	6	Mean
125	0.18	0.23	0.16	0.21	0.18	0.24	0.20
250	0.51	0.52	0.6	0.57	0.55	0.55	0.55
500	1.2	1.13	1.12	1.19	1.18	1.08	1.15
1000	2.09	2.03	2.1	2.15	2.02	1.97	2.06
2000	4.28	4.39	4.33	4.35	4.4	4.41	4.36

Calibration for Epi And 13OH

Concn.	Epi Peak Height Ratio
125	0.07
250	0.17
500	0.3
1000	0.5
2000	1.07

Regression Output:

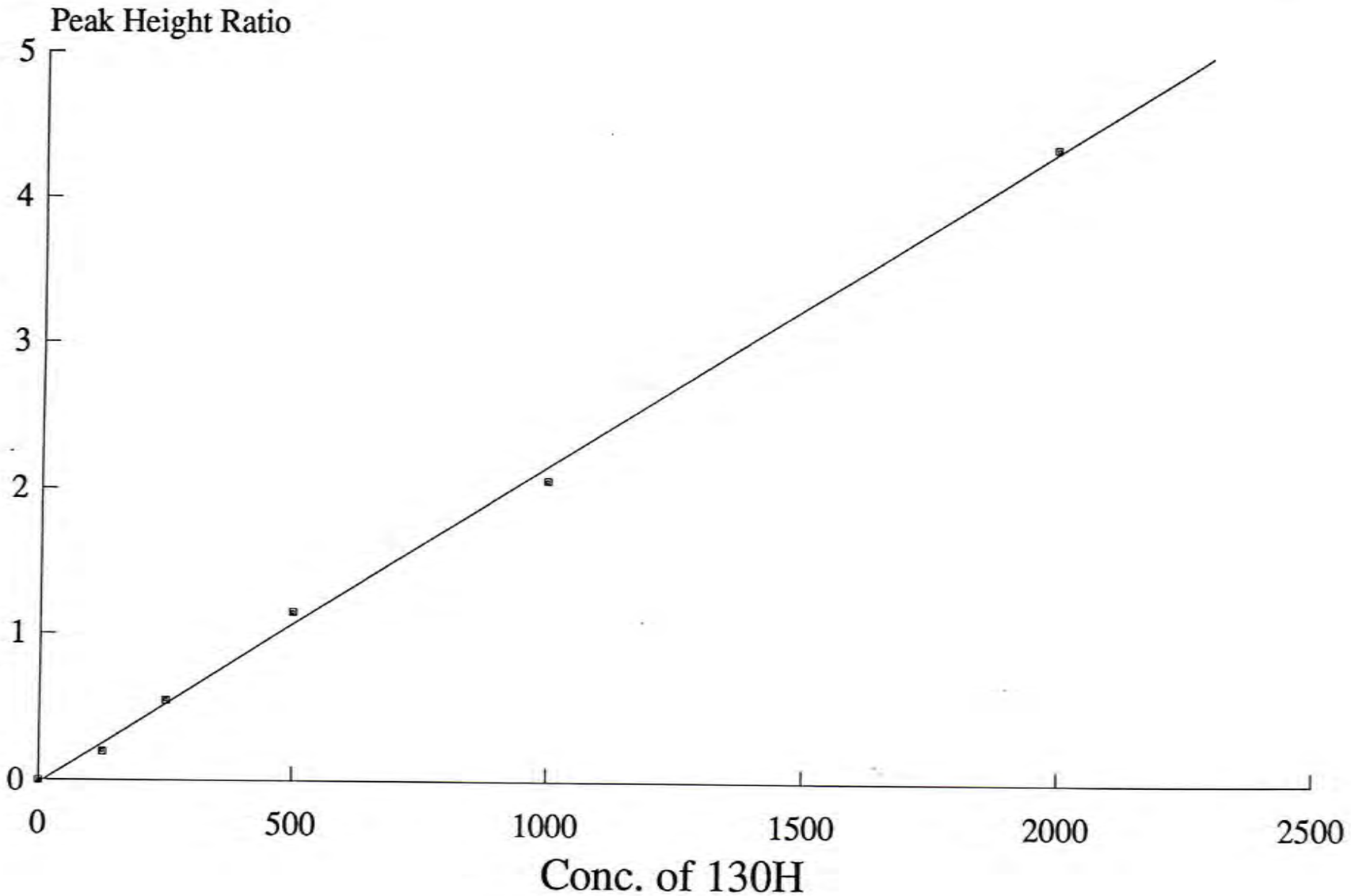
Constant	0
Std Err of Y Est	0.029953
R Squared	0.994287
No. of Observations	5
Degrees of Freedom	4
X Coefficient(s)	0.000533
Std Err of Coef.	0.000012

Concn.	13OH Peak Height Ratio
125	0.2
250	0.55
500	1.15
1000	2.06
2000	4.36

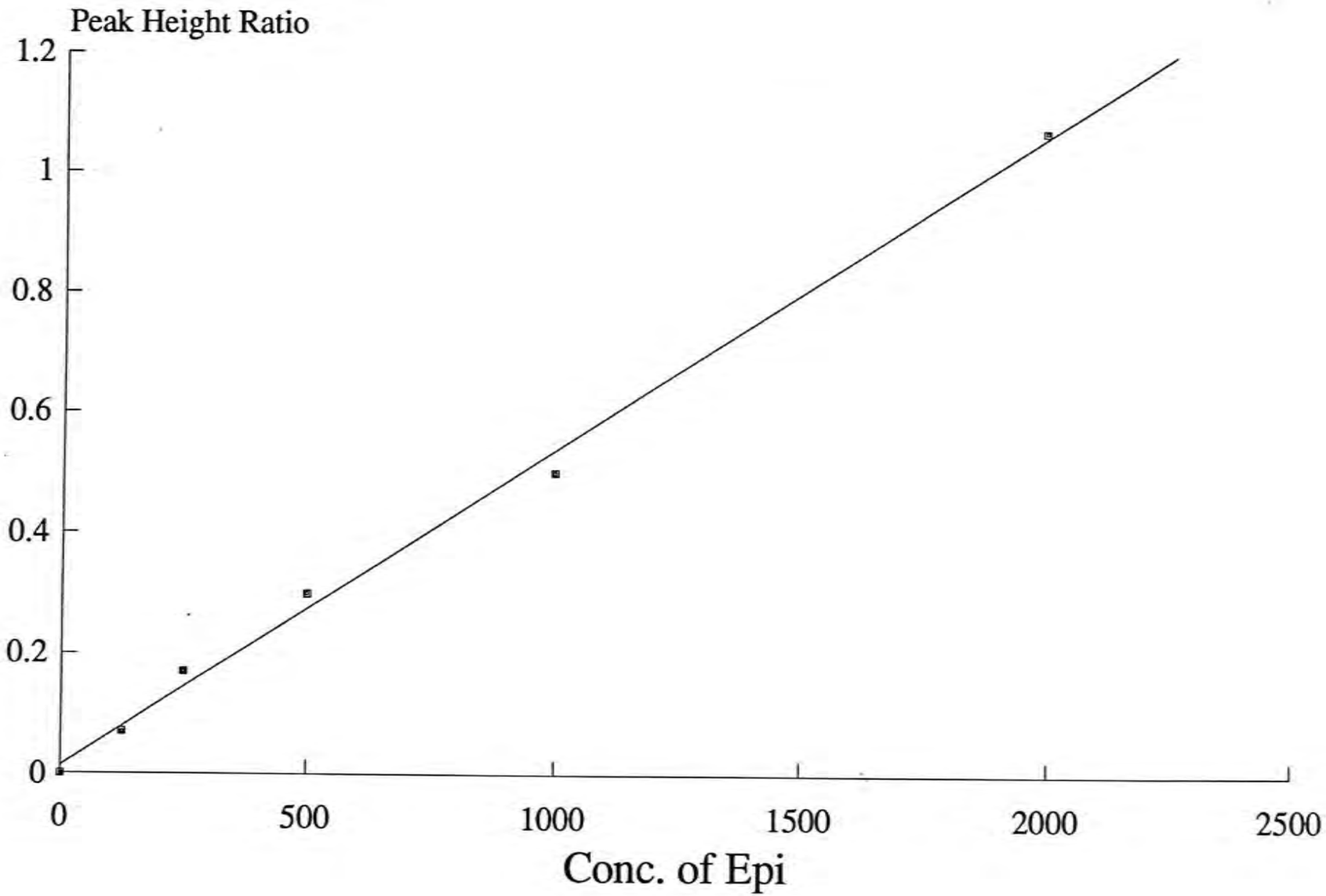
Regression Output:

Constant	0
Std Err of Y Est	0.073289
R Squared	0.998059
No. of Observations	5
Degrees of Freedom	4
X Coefficient(s)	0.002161
Std Err of Coef.	0.000031

Calibration for 130H



Calibration for Epi.



Appendix II

Table (4) - 1

Concentration of Epirubicin (ng/ml) in Emulsion

Time (Hr)\Preparation	Emulsion 1	Emulsion 2	Emulsion 3	Mean ± SD
0	9990.3	9873.2	9520.9	9794.8 ± 244.3
0.5	9080.3	9324.1	8980.2	9128.2 ± 176.9
1	7350.6	7805.2	7547.6	7567.8 ± 228.0
1.5	6880.0	6543.2	6631.8	6685.0 ± 174.6
2	6204.8	6450.2	6713	6546 ± 254.1
2.5	6454.8	6330.2	6783.1	6522.7 ± 234.0
3	6583.1	6331.5	5843.8	6252.8 ± 375.9
3.5	5904.6	5846.3	7105.3	6285.4 ± 710.7
4	6602.6	5200.3	6518.7	6107.2 ± 786.5
4.5	6502.3	5900.5	5454.4	5952.4 ± 525.9
5	5869.3	5501.2	5937.1	5769.2 ± 234.6
5.5	5248.3	5580.2	5604.9	5477.8 ± 199.1
6	5210.3	5840.3	5570.3	5540.3 ± 316.1

Concentration of Epirubicin (ng/ml) in Emulsion

Time (Hr)\Preparation	Emulsion 1	Emulsion 2	Emulsion 3	Mean ± SD
0	0	0	0	0
0.5	0	0	0	0
1	0	0	0	0
1.5	0	350.9	294.7	322.8 ± 39.7
2	0	360.1	302.3	331.2 ± 40.9
2.5	102.3	285.5	299.5	229.1 ± 110.0
3	130.3	320.3	318.6	242.8 ± 99.2
3.5	150.2	370.5	329.8	283.5 ± 117.2
4	201	360.9	297.6	286.5 ± 80.5
4.5	250.2	378.2	342.4	323.6 ± 66.0
5	286.3	390.6	386.9	354.6 ± 59.2
5.5	300.1	410.6	4066.5	372.4 ± 62.6
6	306.8	398.3	363.2	356.1 ± 46.2

Concentration of Epirubicin (ng/ml) in Buffer Solution (Emulsion)

Concentration of Epirubicin (ng/ml) in Solution

Time (Hr)\Preparation	Solution 1	Solution 2	Solution 3	Mean ± SD
0	7502.3	7458.2	7141.1	7367.2 ± 190.0
0.5	6123.5	6850.2	6483.7	6485.8 ± 363.4
1	5304.2	5480.6	6367.7	5711.5 ± 567.0
1.5	4250.2	5023.6	4963.3	4745.7 ± 430.2
2	4050.3	5021.3	5165.5	4745.7 ± 606.6
2.5	5620.1	4950.2	4344.8	4971.7 ± 638.0
3	4350.2	4512.6	4628.8	4497.1 ± 139.7
3.5	3988.5	4280.1	4002.3	4090.4 ± 214.5
4	3850.6	3955	3516.4	3774 ± 229.1
4.5	3650.1	3801.3	4073.4	3841.6 ± 214.5
5	3500	3412.3	5503.5	4138.6 ± 1182.9
5.5	3405.3	3502.4	5508.1	4138.6 ± 1187.0
6	3350.8	3602.6	5792.4	4248.6 ± 1342.9

Concentration of Epirubicin (ng/ml) in Solution

Time(Hr)\Preparation	Solution 1	Solution 2	Solution 3	Mean ± SD
0	0	0	0	0
0.5	217.8	230.2	230	226.0 ± 7.1
1	220.2	229.8	228	226.0 ± 5.1
1.5	278.6	216.9	318.1	161.7 ± 124.2
2	286.4	206.3	320.9	271.2 ± 58.8
2.5	250.3	285.3	278	271.2 ± 18.5
3	289.4	296.3	431.3	3390 ± 80.0
3.5	300.2	308.6	272.6	293.8 ± 18.8
4	320.4	313.3	315.5	316.4 ± 3.6
4.5	320.6	320.8	307.8	316.4 ± 7.4
5	330.8	350.6	335.6	339.0 ± 10.3
5.5	380.6	400.8	439	406.8 ± 29.7
6	420.4	420.1	447.7	429.4 ± 15.8

Concentration of Epirubicin (ng/ml) in Buffer Solution (Solution)

Plasma Concentration of Epirubicin (ng/ml) in 8 rabbits after IV injection of emulsion.

\ Dose Time \ (mg) (min.)\	1 4.4	2 3.45	3 4.8	4 3.9	5 3.85	6 4.35	7 4.4	8 4.5	Mean ± SD
2.5	327.9	88.1	271.8	110.8	905	571.4	194.2	362.4	353.9±270.9
5	77.8	57.8	73.1	107.4	404	250	170.6	267.2	175.9±122.3
10	18.2	23.1	42.7	62.3	204.1	214.3	130.2	180.2	109.4±82.7
15	14.1	22	30.5	27.2	194.4	206.7	64.3	120.6	84.9±79.1
30	10.2			14.8	154.5	200	57.3	54.3	81.8±77.8
60					137.3	171.4	32.3	27.4	85.9±71.9
120					62.3	62.3		12.2	45.6±28.9

Plasma Concentration of Epiribicin (ng/ml) in 8 rabbits after IV injection of solution

\ Dose Time \ (mg) (min.)\	1 4.5	2 4.2	3 4.1	4 3.9	5 4.8	6 4	7 4.5	8 4.3	Mean ± SD
2.5	481.8	957.3	357.1	2521	783.3	362.4	486.4	864.3	851.7±712.8
5	108.6	201.2	151.2	924.4	528.6	275.6	256	424.7	358.8±267.5
10	19	79.3	105.4	404.8	257.1	100.3	172	323.3	182.6±133.6
15	16.7	67	85.7	267.2	200	62.4	74.5	303.2	134.6±106.9
30	11.4	60.9		125	185.7	27.3	30.2	127.8	81.2±65.6
60	8.3	48.8		67.7	52.3	14.2		62.4	42.3±25.1
120		42.7			25.4				34.1±12.2
180		36.6							36.6

Plasma Concentration of Epimbicin (ng/ml) in 10 rabbits after intrahepatic injection of emulsion

\ Dose Time \ (mg) (min.)\	1 4.2	2 4.5	3 4.3	4 4.5	5 4.5	6 4.4	7 3.6	8 3.9	9 4.5	10 4	Mean ± SD
2.5	142.3	103	142.8	127.6	154.3	52.3	132.5	92.6	487.6	204.2	163.9±120.6
5	70.6	28.6	51.9	74.2	42.1	25.5	20.2	17.4	158.9	100.2	58.9±44.3
10	54.3	15.6	13.5	41.5	14.3	19.4	16.1	13.3	95.3	57.3	34.1±27.6
15	36.2	9.5	7.7	34.9	12.7	17.2	9.1	10.2	71	35.2	24.4±20.2
30	32.4	7.7		29.7	11.5	15				17.4	18.9±9.9
60	18.3			16.8	9.3					15.2	14.9±3.9
120	11.7									11.1	11.4±0.4

Plasma Concentration of Epirubicin (ng/ml) in 10 rabbits after intrahepatic injection of solution

\ Dose Time \ (mg) (min.)\	1 4.5	2 4	3 4.45	4 4.2	5 4.5	6 4.3	7 4	8 3.9	9 4.6	10 4.1	Mean ± SD
2.5	243.4	168.4	243.3	326.4	188.8	190.8	132.5	136	928.6	320.4	287.8±235.2
5	127.1	52.7	52.1	82.7	34.8	56.6	39.8	27.4	317.3	180.2	97.1±90.8
10	60.3	15.9	27.4	34.7	15.2	20.9	19.6	13.5	147.6	90.3	44.5±43.7
15	54.3		16.4	23.7	9.7	13.9	12.4	9.3	159.7	42.7	38.01±48.2
30	37.3			23	7.7	12.2	11.3	8.1		12.4	14.7±11.7
60	24.3			17.9	5.3	11.6				10.2	13.9±7.4
120	12.1										12.1